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INSTITUTE FOR MYCORRHIZAL RESEARCH AND DEVELOPMENT

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INSTITUTE FOR MYCORRHIZAL
RESEARCH AND DEVELOPMENT

RESEARCH MISSION

The Institute's mission is to improve artificial regeneration of forest tree seedlings on both conventional and adverse forestation sites by modifying the soil environment through the development of basic and applied technology for the selection, propagation, manipulation, and utilization of ecto- and endomycorrhizal fungi.

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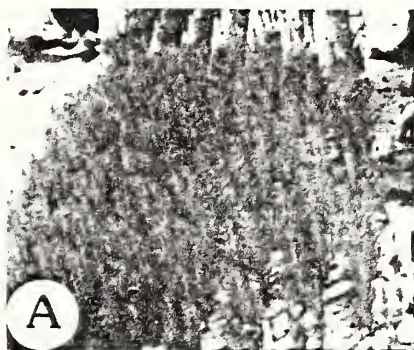
PHOTOS: *A* shows stimulation of pine seedlings after the inoculation of nursery soil with *P. tinctorius*; *B* shows 2-year-old pine seedlings with natural ectomycorrhizae; and *C* shows seedlings with *P. tinctorius* ectomycorrhizae growing on an acid coal spoil.

ECTOMYCORRHIZAE

Many forest trees form symbiotic root associations called mycorrhizae with various soil-inhabiting fungi. In many species, including pines, oaks, beech, birches, firs, eucalyptus, spruces, and hickories, the fungus mainly grows on the outside of the roots, and the association is called ectomycorrhizae. Research here and abroad has shown that ectomycorrhizae are indispensable for the growth of these tree species in their natural environment. Recently, certain ectomycorrhizal fungi, such as *Pisolithus tinctorius,* have been found to be more beneficial to trees than others which may occur naturally. At the Institute, methods are being developed to isolate and propagate *P. tinctorius* and other selected fungi and to inoculate containerized seedlings and those in bare-root nurseries. These seedlings survive and grow faster on certain sites than do the usual nursery seedlings which have ectomycorrhizae formed by naturally occurring fungi.

Fertilizers, pesticides, seedbed densities, tree host genetics, and types of commercial inoculum are among other factors being investigated to determine their influence on the quality and quantity of ectomycorrhizal development. A variety of experiments has been installed on different reforestation and afforestation sites, as well as on reclamation sites, to test and define the benefits of these specific ectomycorrhizal associations to pines and oaks.

A number of private, State, Federal, and international timber-based groups, some industrial fermentation companies, and certain universities, are cooperating with the Institute in this research. Since 1977, 38 states, Mexico, Canada, various parts of Africa, and several Central American countries have engaged in research to determine the areas of practical application.
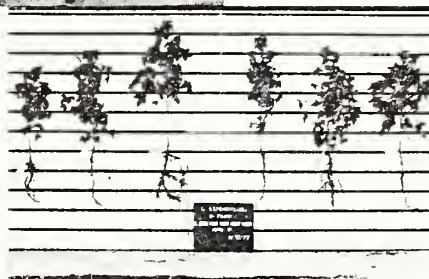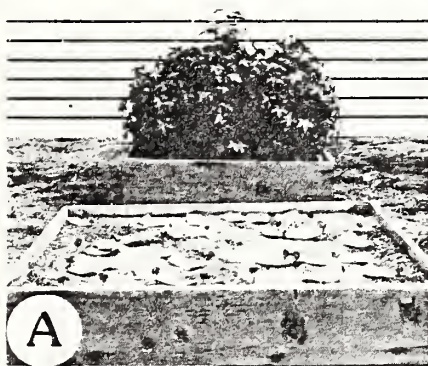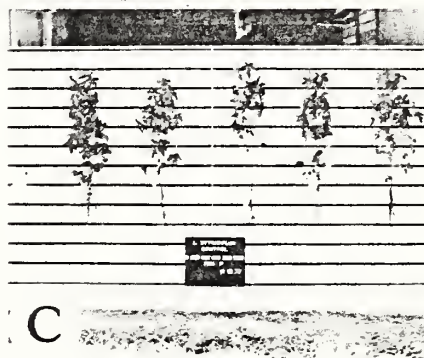
PHOTOS: *A* (foreground) shows severely stunted nonmycorrhizal sweetgum seedlings, and (background) large endomycorrhizal seedlings in low P soil; *B* shows mycorrhizal sweetgum seedlings; and *C* shows nonmycorrhizal seedlings. Both *B* and C show seedlings grown in soil with increasing P rates; 60 p/m of P is needed to substitute for endomycorrhizae.

ENDOMYCORRHIZAE

In many hardwood species of importance in the United States, roots are associated with fungi that penetrate deeply into the roots and form structures called vesicular-arbuscular mycorrhizae. We call these endomycorrhizae VAM for short. Nutrient and water uptake by these trees is enhanced when their roots have VAM, and it is unlikely that sufficient phosphorus required for the basic nutrition of the trees can be obtained from natural forest soils without these root symbionts. Thus, under natural forest conditions, it is not a question of whether these trees can grow better with VAM, but would they survive at all without them. Under cultivated conditions with abundant fertilizer and water, hardwood trees can grow well without VAM and, consequently, the importance of this symbiotic relationship has been overlooked by many plant scientists. Many failures to produce quality hardwood planting stock in tree nurseries are the result of management practices which are unfavorable to the buildup and maintenance of VAM fungal populations. Research is being conducted on the nutritional and environmental factors in nurseries that affect tree host and VAM relationships. The resulting knowledge will help to consistently produce high-quality planting stock needed for the artificial regeneration of these hardwood species.

Our research in the Southern United States indicates that the growth response of sweetgum to a given species of VAM fungus is genetically controlled. We also find that hardy seedlings which have responded well to mycorrhizal associations maintain their superiority when outplanted in the field. Research is being conducted to determine the advantage of grading potential seed orchard sweetgum trees based on progeny performance under specific nursery conditions, such as different species of VAM fungi, phosphorus levels, and root habit. Also underway is the development of a morphological root-grading system for VAM sweetgum seedlings which, it is hoped, will help to determine the selection of seedlings that will have increased competitiveness on regeneration sites.

PHOTOS: *A* shows 3-year-old loblolly pine in fertilized plots; *B* shows seedlings in sludge-amended plots in Copper Basin, Tennessee; and *C* shows 4-year-old loblolly pines with *Pisolithus tinctorius* ectomycorrhizae in fertilized foreground, and sludge-amended background plots on a borrow pit in Aiken, South Carolina.

ADVERSE SITES

Planting sites that have been impoverished by erosion or mechanical removal of topsoil and sites severely disturbed by mining are not conducive to the growth of trees. Chemical and physical alterations of the soil, as well as the manipulation of mycorrhizal fungi on tree seedlings, are needed to convert disturbed sites to maximum forest production. To develop methods for reclamation, research is being conducted in the Southeastern United States on the application of waste materials and other amendments to the soil, intensity and kind of site preparation, the interaction of specific ecto- and endomycorrhizal fungi with different tree species, and nitrogen-fixing plants.

Chemical changes of disturbed sites produced by the addition of fertilizers, lime, or waste materials such as municipal sludge, coupled with specific mycorrhizae on seedling roots, show promise in improving plant production on adverse sites. Sewage sludge has been particularly beneficial on poor sites, such as borrow pits and the Tennessee Copper Basin, where all topsoil was removed or eroded. Where it is not feasible to transport heavy, bulky amendments, compressed sewage sludge tablets or small quantities of slow-release fertilizer placed in the closing hole at the time of planting the seedling can induce rapid growth.

Physical changes brought about by deep ripping or subsoiling allow better soil aeration. The interaction of subsoiling intensity (varied depth and spacing), sludge, and specific ectomycorrhizae on pine seedling roots is currently being studied.

In the Southeast, particular attention is being paid to the adaptability of different tree species and hybrids on harsh sites after subsoiling or the use of soil amendments. Early growth stimulation of longleaf pine by specific ectomycorrhizae and amendments on its native, deep sandy soil is also being investigated.

Those who plant, maintain, and develop forests must realize that trees are first a root crop and then a fiber crop. The soil environment includes a variety of biological, physical, and chemical factors which exert a beneficial or a harmful influence on the survival and growth of forest trees. Practical and biologically sound methods must be found to enhance the beneficial factors and to eliminate or modify the harmful ones. We can no longer afford the time, energy, or money to simply "plant and pray." Only through sound research on soil factors and careful planning can we achieve practical goals. The Institute is one example of the research efforts of the Forest Service to assure the regeneration of tomorrow's forests.

Proceedings

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ECTOMYCORRHIZAE: BENEFITS AND PRACTICAL APPLICATION IN FOREST TREE NURSERIES AND FIELD PLANTINGS

Charles E. Cordell and Donald H. Marx[1]

Abstract.--During the past several years, the U. S. Forest Service has been conducting extensive research and field application studies with several cooperating forest agencies concerning the practical application of selected ectomycorrhizal fungi to bareroot and container seedling nurseries. Significant positive results concerning increased seedling growth and quality in the nursery along with increased tree survival and growth in field plantings have been obtained on a wide variety of conifer species and planting sites. Several alternative methods are presented concerning the practical inoculation of nursery seedbeds with ectomycorrhizal fungus inoculum.

Additional key words: Pisolithus tinctorius, fungus inoculum, conifer host species, seedling growth and quality, tree survival and growth, bareroot nurseries, container nurseries.

INTRODUCTION

During the past several years, researchers at the Mycorrhizal Institute in Athens, Georgia, and Pest Management specialists in Atlanta, Georgia, both with the U. S. Forest Service, have been conducting extensive ectomycorrhizal research and field application studies with several forest agencies (Anderson and Cordell, 1979). This work has centered around one ectomycorrhizal fungus, Pisolithus tinctorius (P.t.).

Since 1977, a national evaluation has been in progress to test the effectiveness of different formulations of a commercial P.t. inoculum on selected conifer seedling species. Abbott Laboratories, 36 Oakwood Road, Long Grove, Illinois, a major pharmaceutical company, is developing techniques for the commercial production of P.t. inoculum. During the past 3 years, over 80 bareroot nursery tests have been conducted in some 38 states. Eighteen companion container nursery seedling evaluations have also been conducted in nine states (including Hawaii) and Canada.

The objective of both the bareroot and container seedling evaluations was to compare the effectiveness of the Mycorrhizal Institute and Abbott Laboratories P.t. inocula for ectomycorrhizal formation, seedling growth and quality in the nursery, and tree survival and growth in subsequent field outplantings. This is a cooperative project between the Institute for Mycorrhizal Research and Development - Athens, Georgia, Forest Pest Management - Southeastern Area - State and Private Forestry - Atlanta, Georgia, and various other U. S. Forest Service, state, industry, and university agencies.

Results obtained to date suggest that P.t. may have practical application for a variety of conifer and some hardwood seedling species produced in bareroot and container nurseries.

BENEFITS OF P. TINCTORIUS ECTOMYCORRHIZAE

Bareroot Nurseries

Research and nursery field evaluation results have repeatedly demonstrated that P.t. can be successfully artificially inoculated into fumigated nursery seedbeds (Fig. 1) (Marx, et. al., 1976, Marx and Artman, 1978). Successful seedbed inoculations have been obtained in a variety of nurseries with various conifer species and some oaks. The P.t. inoculum produced by the Mycorrhizal Institute has consistently shown positive nursery seedling benefits (Fig.2). Results obtained from inoculations with Institute inoculum in 12 southern and central nurseries during 1978 showed seedling fresh weights increased an average of 26 percent and seedling culls decreased an average of 26 percent as compared with untreated control seedlings (Fig. 3) (Cordell and Marx, 1979). Thus far, the different formulation of the commercial P.t. has not been as consistent as the Institute P.t. in either the formation of P.t. ectomycorrhizae or on seedling growth and quality. Some inoculum batches have been highly effective. Research in 1980 is aimed at rectifying these formulation problems. Preliminary results, however, from the 1980 studies are most promising.

Container Nurseries

Results obtained from the 1978 container seedling studies with both the Institute and commercial P.t. inoculum were highly encouraging. The Institute P.t. produced an average of 40 percent P.t. ectomycorrhizae on seedling feeder roots. The commercial P.t. produced an average of 20 percent P.t. ectomycorrhizae. Both P.t. inoculum sources produced some P.t. ectomycorrhizae on 100 percent of the seedlings on several conifer species. In addition, there was almost a 300 percent increase in total ectomycorrhizae development on the Institute P.t.-treated seedlings. Over a 200 percent increase occurred on the commercial P.t.-treated seedlings, as compared with the uninoculated seedlings which must rely on natural occurring fungi (i.e. Thelephora terrestris) for ectomycorrhizal development.

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Figure 1. P. tinctorius (P.t.) fruiting body (puff ball) pro-
duced in nursery seedbed 3-4 months following inocu-
lation.
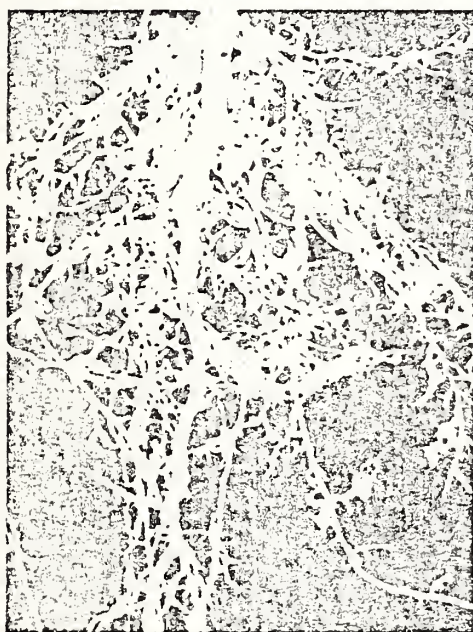
A   B

Figure 2.- Roots of loblolly pine seedlings artificially inocu-
lated with P.t. (A) and uninoculated (B) in a Georgia
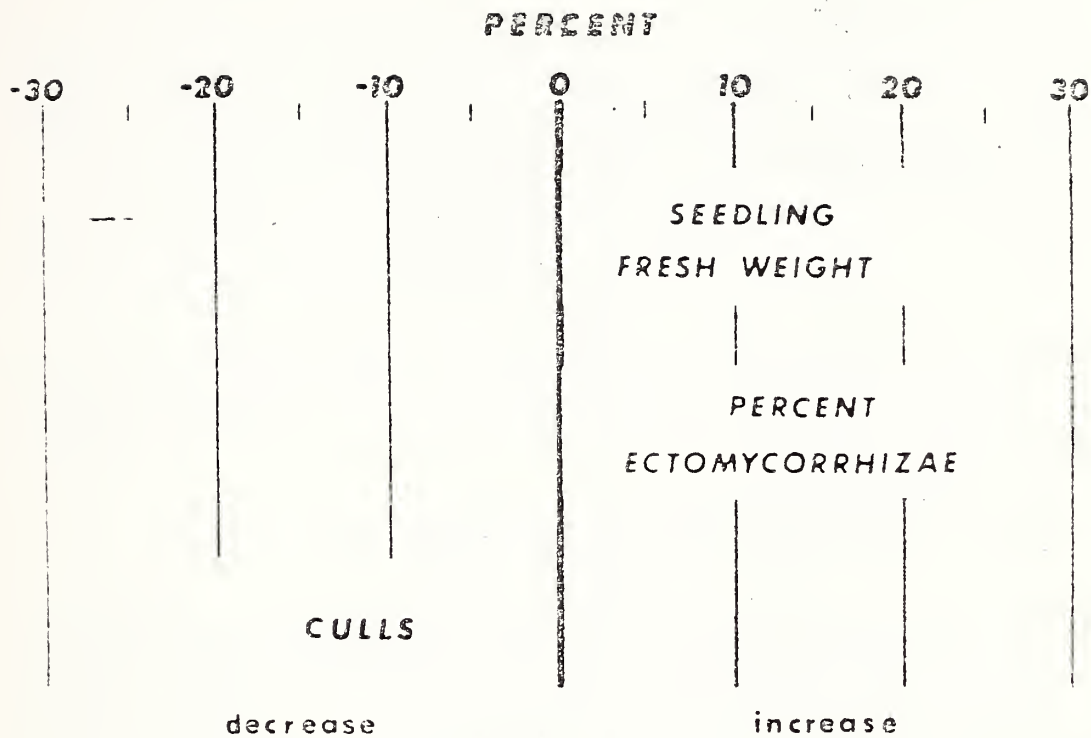nursery.

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Figure 3. Nursery seedling benefits (increased quality and decreased culls) obtained from Institute P.t. mycelium artificial inoculations in 12 southern bareroot nurseries in 1978.

Field Plantings

Outplanting tests were made on a variety of forestation sites—routine, coal and kaolin spoils, etc.—in scattered locations in the United States prior to the commercial P.t. inoculum test (Berry and Marx, 1978: Cordell and Marx, 1977; Marx, et. al., 1977). Significant increases occurred in survival and tree growth after as long as 6 years in the field, on some sites. Increases in tree survival and growth of over 25 percent have been obtained. The field forestation benefits have been consistently higher on the poorer quality planting sites.

Initial outplantings with the commercial P.t. were established during the spring of 1979. To date, over 15 outplantings have been established. Additional outplantings are scheduled for a variety of conifer species and planting sites across the United States.

PRACTICAL APPLICATION OF P. TINCTORIUS IN BAREROOT AND CONTAINER NURSERIES

The most practical and effective means of using P.t. is by inoculating either bareroot nursery seedbeds or containers before seeding. Best results have also been obtained following effective methyl bromide soil fumigation of the nursery seedbeds. Seedling container mixes can be easily and effectively treated with the inoculum before seeding (Ruehle and Marx, 1977).

The following possible alternatives are available for consideration with P.t. inoculations in bareroot nurseries:

1. Commercial inoculum broadcast on seedbed surface with fertilizer spreader equipment and rototilled in with a bed shaper before seeding. This method is simple and does not require special equipment. However, it requries a larger, much more expensive volume of P.t. inoculum.

2. P.t. spores mixed with hydromulch and applied to nursery seedbeds immediately after seeding. This method has primary advantages similar to 1, above, other than the need for a hydromulcher. However, there are several disadvantages to this inoculation method. First, a large volume of P.t. spores is required. Second, the spore germination lag time gives a competitive advantage to other soil fungi. Results to date have shown the P.t. spore inoculum to be less effective than laboratory-grown P.t. inoculum (mycelium with spores) in nursery seedbeds. However, results obtained from a P.t. spore hydromulch inoculation study in Oklahoma were very effective (Marx, et. al., 1979).

3. P.t. spores encapsulated seed treatments. Although still under
    study, preliminary results in some nurseries have been very
    promising. The advantages and disadvantages of this method are
    similar to other P.t. spore inoculation methods. In coopera-
    tion with the Mycorrhizal Institute, an Alabama forestry firm
    is developing plans and techniques for the production of P.t.
    spore-encapsulated seed for a variety of conifers.

4. Commercial inoculum applied in nursery seedbed drill rows with
    an ectomycorrhizal fungus applicator-tree seeder. During 1979
    a standard nursery seed planter was modified by the USDA, Forest
    Service for the simultaneous application of P.t. inoculum and
    seedbed sowing in nursery seedbeds (Fig. 4).
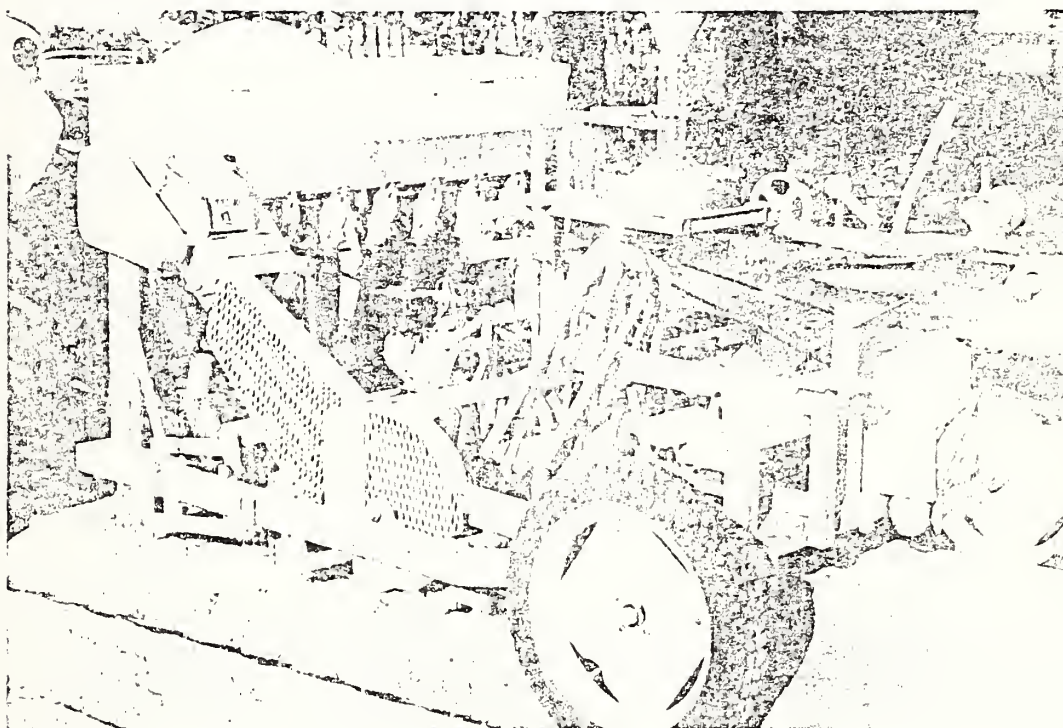
Figure 4. P.t. ectomycorrhizal fungus applicator-nursery tree
           seeder.

The ectomycorrhizal fungus applicator-tree seeder is being
tested in four southern nurseries during 1980. If effective in
promoting P.t. development on seedling feeder roots, such a ma-
chine should be highly useful in forest tree nurseries. A major
advantage of this seedbed row-drilled technique would be a much
lower volume for P.t. inoculum (67 percent less than seedbed broad-
cast method) as well as substantial savings in time, labor, and
other costs. The additional machine requirement is a primary dis-
advantage. However, most existing nursery seeders could be modi-
fied, at relatively minimal cost, to apply the inoculum.

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The four inoculation alternatives are presented only for consideration in making bareroot nursery cultural management plans and decisions. The advantages and disadvantages with each method could change with further research and nursery evaluation studies. Consequently, none of these methods can presently be recommended for routine use. Most likely, one or more alternative methods will be most effective and practical in a particular nursery depending on the prevailing biological, environmental, and economical conditions.

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Improved formulations of Abbott inoculum (MycoRhiz[R]) of *Pisolithus tinctorius* (Pt) in the final phase of testing this past year was found to be effective. A machine developed by the U.S. Forest Service for dispensing MycoRhiz[R] in nursery soil has been tested. Mechanized inoculum application permits uniform distribution and proper depth placement; this allows for significant reduction in the volume per acre needed to produce bare-root pine seedlings well colonized with Pt.

Basidiospore inoculum can be successfully used to tailor container-grown pine seedlings with Pt ectomycorrhizae. Recent greenhouse and microplot nursery tests with encapsulated seed (basidiospores are impregnated in the encapsulating material surrounding the seed) show considerable promise. Conventional nursery and field tests employing this type of seed encapsulation are being planned.

Pine genetics and ectomycorrhizal technology have been integrated in a current field study in South Carolina. Ten half-sib families of loblolly pine with three ectomycorrhizae treatments (*Pisolithus*, *Thelephora* and control) were grown in our experimental nursery and outplanted on a routine reforestation site in South Carolina.

Means of utilizing mycorrhizal technology in developing countries have been initiated in Liberia and Morocco. A current study in Liberia on an area having over 90 percent of its annual rainfall during four months has revealed after one growing season that *Pinus caribaea* exhibits significant improvement in survival and growth when colonized with Pt at planting. Greenhouse and microplot trials at the Mycorrhizal Institute have shown that conifers native to Morocco (*Pinus halepensis, P. Pinaster* and *Cedrus atlantica* are readily colonized with a pine isolate of Pt. Plans are being made for a Moroccan forester, currently studying our techniques in Athens, to install field studies in Morocco next year.

Research is continuing on phosphorus-endomycorrhizae interactions on sweetgum in nursery trials and field plantings. Current field trials are comparing sweet-gum seedlings produced to plantable size in the nursery with high levels of P to those produced to the same size employing inoculation with endomycorrhizal fungi and low P. Family differences in root system configuration are also being examined in these interaction trials. Apparently root grade, P fertility, and mycorrhizal infection all interact to influence field survival and early seedling growth.

Studies on reclamation of borrow pits are continuing. One study in Aiken, South Carolina, involves nine degrees of subsoiling with either one-fourth inch broadcast-disked sewage sludge or 1,000 pounds per acre of 10-10-10 fertilizer and dolomitic limestone. Over 4,400 loblolly pines with abundant Pt ectomycorrhizae were planted last winter in this study.

Tropical Mycorrhiza Research

Edited by
PEITSA MIKOLA

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2 Ectomycorrhizal fungus inoculations: a tool for improving forestation practices

D. H. Marx

Introduction

The need of many species of forest trees for ectomycorrhizal associations was initially observed when attempts to establish plantations of exotic pines routinely failed until the essential fungi were introduced (Briscoe 1959; Clements 1941; Gibson 1963; Hatch 1936; Kessell 1927; Madu 1967; van Suchtelen 1962). The need of pine and oak seedlings for ectomycorrhizae has also been convincingly demonstrated in the afforestation of former treeless areas, such as the grasslands of Russia and the Great Plains of the United States (Goss 1960; Hatch 1937; McComb 1938; Rosendahl and Wilde 1942; Shemakhanova 1962; White 1941).

The primary purpose for inoculating with symbiotic fungi in world forestry is to provide seedlings with adequate ectomycorrhizae for planting in man-made forests. Such treatment has proven essential in forestation of cutover lands and other treeless areas, introduction of exotic tree species, and reclamation of adverse sites such as mining spoils. Use of ectomycorrhizal fungi can be of major significance to artificial regeneration.

Most research on inoculation with ectomycorrhizal fungi has been based on two working premises. First, any ectomycorrhizae on roots of tree seedlings is far better than no ectomycorrhizae at all. Success in correcting these deficiencies has contributed greatly to our understanding of the importance of ectomycorrhizae to trees, especially as they relate to the establishment of exotic forests. Secondly, some species of ectomycorrhizal fungi under certain environmental conditions are more beneficial to trees than other fungal species. Much more research should be aimed at selecting, propagating, manipulating, and managing the more desirable fungal symbionts to improve tree survival and growth.

The majority of past work on inoculation with ectomycorrhizal fungi has been done in nurseries for the production of bare-root or containerized tree seedlings. Most future work with inoculations will undoubtedly continue to concentrate on seedling production. However, the inoculation of seed for direct seeding operations could become a very important alternative to planting seedlings, especially on the more remote sites or those of very rough terrain.

Trappe (1977), Mikola (1973), and others (Bowen 1965; Imshenetskii 1955; Shemakhanova 1962) have thoroughly reviewed past work on ectomycorrhizal inoculations. I, therefore, am free to discuss selected reports as they relate to specific procedures and concentrate on recent published and unpublished work on pure culture manipulation of ectomycorrhizal fungi.

Ectomycorrhizae are formed by fungi belonging to the higher Basidiomycetes (mushroom and puffball group), Ascomycetes, and zygosporic Phycomycetes of the Endogonaceae (Gerdemann and Trappe 1974; Trappe 1962, 1971). The host plants of these fungi are predominantly trees belonging to the Pinaceae, Fagaceae, Betulaceae, Salicaceae, Juglandaceae, and other families (Meyer 1973).

Many species of fungi may be involved in the ectomycorrhizal associations of a forest, a single tree species, an individual tree seedling, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza (Zak and Marx 1964). Even as a single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots, a single fungus species can also enter into mycorrhizal association with numerous tree species. Some fungi are apparently host specific; others have broad host ranges and form mycorrhizae with members of numerous tree genera in diverse families (Trappe 1962).

The majority of reports on inoculations with ectomycorrhizal fungi involve Basidiomycetes on pines, oaks, and eucalypts. Several types of natural and laboratory produced inocula and methods of application have been used through the years. Many of these procedures have proved successful, others have not. Frequently, conflicting results are encountered.

Natural dissemination of spores

No ectomycorrhizal fungus in a natural environment has been shown to complete its life cycle in the absence of mycorrhizal association (Hacskaylo 1971). There also is no evidence to suggest that these fungi grow saprophytically in a natural forest soil (Shemakhanova 1962). However, once root infection has taken place extramatrical growth of mycelium from roots into large soil volumes is not unusual. Sporophores may occur dozens of metres from the above-ground parts of the tree host.

Most ectomycorrhizal fungi produce sporophores containing numerous spores. These spores can be disseminated great distances by wind, rain, insects, small animals, etc. The greater the density and closeness of the ectomycorrhizal tree hosts to the seedling producing areas, the greater the chances are for rapid natural ectomycorrhizal

development on the seedlings. In the southern United States, ectomycorrhizae appear in the spring on nursery-grown pine seedlings as early as six to eight weeks after seed germination. This occurs even in nursery soil fumigated just a few days prior to seeding because these nurseries are usually surrounded by dense stands of pine and oak which support abundant sporophores of mycorrhizal fungi. However, dry or cold weather often influences the fruiting habits of these fungi causing erratic spore production and dissemination. As mentioned by Trappe (1977), although heavy rains during the fruiting season stimulate fruiting, these rains can also restrict dispersal by washing spores from the air near their source.

The cultural procedures used to produce seedlings in bare-root or container nurseries create environmental conditions which select certain mycorrhizal fungi adapted to these conditions. In the southern United States, as well as other parts of the world, *Thelephora terrestris* appears to dominate the roots of most pines and oaks grown in nursery soil (Marx, Bryan, and Grand 1970; Mikola 1970; Weir 1921) and in containers (Marx and Barnett 1974).

In the southern United States, spore dissemination begins shortly after the nursery soil has been fumigated and seeded (usually from March to May) when *T. terrestris* produces sporophores and abundant spores in adjacent forests. These spores are carried by the wind to the fumigated soil, leach through the soil a few centimetres, and rapidly colonize the seedling roots.

This early colonization of seedling roots by *T. terrestris* can preclude colonization by other fungi which produce spores later in the year. These other fungi may form some mycorrhizae on the seedling roots later in the growing season, but only rarely do they dominate the roots. To maintain superiority on the roots, a fungus that appears first on seedlings must form mycorrhizae on short roots as rapidly as the short roots are produced. If it fails to do so other available fungi will infect these new roots. *Thelephora terristris* and the other fungi which naturally occur and dominate seedling roots in nurseries have the capacity to spread rapidly under nursery conditions.

In nurseries in the United States and many other parts of the world, production of tree seedlings (particularly pine, oak, spruce, fir, and eucalyptus) is not seriously affected by deficiencies of ectomycorrhizae. In 1975, for example, there were over 1.2×10^9 coniferous tree seedlings produced in 190 nurseries in the United States. It is highly unlikely that these seedlings would have reached plantable size if they had not had adequate mycorrhizae. Ectomycorrhizal fungus deficiencies in properly managed tree nurseries in the United States, therefore, are rare.

The few mycorrhizal deficiencies reported in the United States in

recent times (Marx, Morris, and Mexal 1978; Trappe and Strand 1969) have appeared in newly established nurseries in which the soil was fumigated to control weeds and pathogens. Fumigation also eliminates residual inoculum of ectomycorrhizal fungi.

Not all deficiencies or the erratic occurrence of ectomycorrhizae on seedling roots are due to the absence of inoculum in soil. Excessive use of soluble fertilizers can reduce susceptibility of roots to infection (Bowen 1973; Marx, Hatch, and Mendicino 1977). In addition to fumigants, certain fungicides can significantly reduce or eradicate inoculum in soil and cause a deficiency of mycorrhizae on seedling roots (Hacskaylo and Palmer 1957; Iyer, Lipas, and Chesters 1971; Wojahn and Iyer 1976). However, it should be pointed out that certain fungicides can also stimulate development of ectomycorrhizae (Marx and Bryan 1969a; Powell, Hendrix, and Marx 1968), as can the application of certain nutrients to the soil which either stimulate residual inocula or host-root suscpetibility (Bowen 1973).

Natural inoculation of containerized seedlings grown in sterile or near-sterile potting mix is undoubtedly from airborne spores, but in many instances it is very erratic (Trappe 1977). In the southern United States pines are grown in a variety of containers for approximately three to four months (Balmer 1974). Natural ectomycorrhizal development on these seedlings is often erratic because they are watered and fertilized heavily to obtain the fastest possible growth in the shortest possible time. Unfortunately, these conditions induce high shoot-root ratios and low incidences of mycorrhizae on pine, both of which are thought to be undesirable for best field performance of seedlings (Marx and Barnett 1974; Marx, Hatch, and Mendicino 1977; Ruehle and Marx 1977). To ensure good ectomycorrhizae on containerized seedlings, procedures for inoculation with specific fungi and providing nutrients and water must be perfected (Ruehle and Marx 1977; Trappe 1977).

Cultural practices in nurseries influence the incidence of specific ectomycorrhizal fungi on tree seedlings. Levisohn (1965) reported that for many years *Suillus bovinus* was the only mycorrhizal fungus on seedlings of *Picea sitchensis* in certain nurseries in England. These nurseries were not fumigated and the soils were heavily composted each year with a variety of organic residues. The *S. bovinus* mycorrhizae soon disappeared from roots of *P. sitchensis* after outplanting unless the outplanting site was also heavily composted with organic matter. These results suggest that this fungus is not adapted to soils with low levels of organic matter. During subsequent years the nursery expanded and began to use different methods of fertilization in addition to amending the soil with organic residues. Associated with those cultural changes in the nurseries was the appearance of *Rhizo-*

pogon luteolus. Eventually this fungus dominated the spruce seedlings in the nursery, and thereafter *S. bovinus* occurred only rarely.

Soil or humus containing natural inoculum

The easiest and simplest method for eliminating ectomycorrhizal fungus deficiencies on seedlings in nurseries is to apply soil, humus, or duff containing mycorrhizae and associated mycelium. It is by far the most commonly used method to ensure consistent development of mycorrhizae (Mikola 1973). This form of inoculum can be collected from natural forests, plantations, or established tree nurseries. It is a very reliable method if done properly. The use of soil inoculum to propagate and maintain specific mycorrhizal fungi is unusual, but soil from truffle producing areas was used to inoculate seedlings in new areas (Malencon 1938). The success of this effort is unknown. In the Soviet Union (Shemakhanova 1962), soil containing mycorrhizae is routinely placed in holes prior to planting acorns for regeneration of oaks in shelterbelts. These areas of the Soviet Union are not devoid of ectomycorrhizal fungi, but apparently soil inoculation increases survival and early growth of the oak seedlings. The purpose of inoculation is to introduce new fungi into the area and enhance development of mycorrhizae on seedlings. The latter point is considered critical for artificial regeneration of oaks in Russia.

Mikola (1970) discusses in detail the various practices used in tropical and subtropical countries to ensure mycorrhizal development on nursery seedlings raised in various containers (pots, wooden boxes, plastic tubes, Swaziland beds, etc.). In most instances, 10 to 20 per cent of the container mixture is topsoil or humus containing mycorrhizae collected from a healthy pine plantation or established nursery. In order to conserve soil inoculum a small quantity of soil is sometimes added to the base of individual seedlings in containers.

A major drawback to the use of soil or humus as inoculum is that the specific fungi in the mixture cannot be controlled. Also there is no assurance that the chosen soil inoculum contains the most desirable fungi for the tree species being produced. The large volumes of soil needed to inoculate a nursery creates a logistics problem since 10 per cent of the volume of soil is currently recommended to assure adequate inoculation of nurseries (Mikola 1973). This volume of mycorrhizal soil can be extremely large in a nursery covering many hectares. Soil inoculum may contain a variety of harmful microorganisms and noxious weeds in addition to the ectomycorrhizal fungi. Some of these micro-organisms may not be potentially harmful only to the tree seedling crop (Mikola 1973) but possibly to nearby agricultural crops (Marx 1975).

Ectomycorrhizal seedlings or excised ectomycorrhizae

Tree seedlings with ectomycorrhizae or excised mycorrhizae have been used as inoculum for new seedling crops. Chevalier and Grente (1973) were able to successfully establish the truffle fungus *Tuber melanosporum* in nursery beds from seedlings previously inoculated with this fungus. New seedlings growing adjacent to the pre-inoculated seedlings formed *T. melanosporum* ectomycorrhizae. Mikola (1973) discussed the Indonesian technique of inoculation. Seedlings with abundant mycorrhizae are planted at one to two metre intervals in new seedbeds. This technique is highly successful in forming ecto-mycorrhizae on seedlings of *Pinus merkusii*. Levisohn (1956) used surface-sterilized pine roots with *Rhizopogon luteolus* mycorrhizae to successfully form mycorrhizae on and stimulate growth of *Pinus contorta* seedlings in pots. Ekwebelam (1973) inoculated *Pinus caribaea* var. *hondurensis* and *P. kesiya* by growing them for three months in polyethylene bags filled with sterile sand containing excised mycorrhizae formed by *Rhizopogon luteolus*. The typical white coralloid ectomycorrhizae usually associated with *R. luteolus* was observed on roots of the seedlings within one month of germination. Ekwebelam did not mention non-inoculated control seedlings in his experiment, but in his previous experiments non-inoculated seedlings grown under similar conditions in this area of Africa apparently remained free of ectomycorrhizae.

Procedures using seedlings with ectomycorrhizae or excised mycorrhizae may be useful in propagating a specific fungus if soil conditions maintained in the nursery favour the introduced fungi and not those that occur naturally. Unless the introduced fugus is more adapted to nursery conditions than the naturally occurring ones, the desired fungus will eventually be displaced from roots.

Sporophores and spores

According to Trappe (1977), the first attempts to use specific fungi to form mycorrhizae on seedlings dates back to the eighteenth century. Sporophores of truffle fungi were added to planting holes of oak seedlings in new plantations in attempts to enhance truffle production (Malencon 1938). These inoculations took place nearly 75 years before the term 'mycorrhiza' was coined and over 100 years before the true nature of ectomycorrhizal associations was demonstrated. Unfortunately there is no way of determining to what degree these inoculations were successful. Sporophores of various ecto-mycorrhizal fungi, such as *Pisolithus tinctorius* and *Rhizopogon luteolus*, have been dried and/or chopped into small pieces and used

to infest soil successfully (Donald 1975; Fontana and Bonfante 1971; Mullette 1976). Fresh sporophores have also been added to soil inoculum prior to its use in nurseries to enhance the infective capacity of the soil (Mikola 1973). Inoculum composed of whole or chopped sporophores is basically nothing more than spore inoculum, since the vegetative matrix of the sporophores undoubtedly decomposes shortly after incorporation into soil.

Portugal

In 1970, Azvedo (personal communication) began developing a technique of seed inoculation using dried sporophores of different ectomycorrhizal fungi. Sporophores of *Amanita muscaria*, *A. phalloides*, *Boletus granulatus*, *B. scaber*, *Hydnellum zonatum*, *Lactarius delicious*, *L. chryzoreus*, *Lepiota procera*, *Russula cyanoxantha*, *Sarcodon imbricatum*, and *Tricholoma terreum* were collected fresh and dried carefully in the laboratory for one week. They were then transferred to a dessicator maintained at 30 °C for a few more days to complete the dehydration, crushed into a fine powder, and stored in sealed polyethylene bags. Seeds of *Pinus pinaster* were moistened with water and coated with the dried inoculum. After six to eight months in greenhouse tests using steamed soil in pots, *A. muscaria*, *R. cyanoxantha*, *S. granulatus*, and *H. zonatum* were found to be the most efficient fungi in forming typical ecto- and ectendomycorrhizae on *P. pinaster*. In another test, *R. cyanoxantha*, *T. terreum*, and *B. granulatus* formed the most ecto- or ectendomycorrhizae on *P. pinaster* after three months in Japanese paper pots. Control seedlings from non-inoculated seed, in most instances, remained free of any type of mycorrhiza. Azevedo states that this dried form of inoculum remains viable for four to five years when properly stored. Again, we can assume that the functional portion of the dried sporophores is basidiospores and not mycelium. These dried basidiospores of various fungi survived considerably longer than basidiospores of *Rhizopogon luteolus* in other experiments.

Australia

Pryor (1956) added basidiospores of *Scleroderma flavidum* to heat-sterilized soil in pots. Abundant ectomycorrhizae formed on roots and growth of *Eucalyptus dives*, *E. pauciflora*, and *E. macrorrhyncha* was stimulated. From these results he concluded that the absence of ectomycorrhizae on these *Eucalyptus* spp accounted for regeneration failures in Iraq and other parts of the world.

Theodorou (1971) found that inoculation of seeds of *Pinus radiata* with freshly harvested basidiospores of *R. luteolus* was an easy and effective way of introducing mycorrhizal fungi into both sterile and

non-sterile soil (mycorrhizal fungus deficient) in pots and in the field. This technique involved soaking surface-sterilized seeds of *P. radiata* in a sterile water suspension of basidiospores which coated each seed with approximately 1.9×10^6 spores. Theodorou found that more mycorrhizae formed on seedlings grown in sterilized soil than on those grown in non-sterile soil. He concluded that sterilization of soil enhanced mycorrhizal development by eliminating soil organisms deleterious to *R. luteolus*. Theodorou and Bowen (1973) later found that spores from freeze-dried sporophores of *R. luteolus* could be used to inoculate seed. Seed coated with basidiospores could be dried and stored ($2\,^\circ$C) for one month. They found that spore numbers must be increased by up to 100 times with freeze-dried spores and up to ten times with spores air-dried for two days to obtain ectomycorrhizal development on *P. radiata* seedlings equal to that of freshly collected basidiospores. Apparently freeze- and air-drying kills or inhibits germination of a substantial number of these basidiospores.

In pot studies Lamb and Richards (1974a,b) found that chlamydospores of three unidentified fungi were generally not as effective as basidiospores of *Rhizopogon luteolus, Suillus granulatus*, or *Pisolithus tinctorius* in forming ectomycorrhizae on *Pinus radiata* in natural soils lacking ectomycorrhizal fungi. The effectiveness of the different types of spore inocula, however, was improved by increasing inoculum density of the fungi or by increasing the amount of available phosphorus in the soils to 40 kg/ha. This stimulating effect of phosphorus is somewhat surprising since Mullette (1976) reported that basidiospores, i.e. crushed sporophores of *P. tinctorius*, would not form mycorrhizae on *Eucalyptus gummifera* in sterile quartz containing more than 3 kg of available P/ha (5 p.p.m.).

South Africa

Donald (1975) added air-dried and ground sporophores of *Rhizopogon luteolus* to fumigated soil in South Africa prior to seeding *Pinus radiata*. After eight months, seedlings from inoculated soil in one nursery had abundant white ectomycorrhizae with loose mycelium radiating from them. Sporophores of *R. luteolus* occurred in the inoculated beds and were associated with the white mycorrhizae. Donald concluded that the functional component of the dried sporophores was basidiospores and that they (4.4×10^7 spores per m^2 of soil surface) can be used as inoculum to form mycorrhizae on *P. radiata* in a conventional tree nursery.

United States

In recent years basidiospores of *Pisolithus tinctorius* have been used

in a variety of nursery and container tests on pines in the southern United States. Marx and Bryan (1975) added freshly collected basidiospores of *P. tinctorius* to fumigated soil in nursery microplots at a rate of $1.3 \times 10^{10}/m^2$ around two-month-old seedlings of *Pinus taeda.* *Pisolithus* formed approximately half of all the ectomycorrhizae on seedlings by the end of the growing season. At the time of soil infestation these seedlings had a few ectomycorrhizae formed by naturally occurring *Thelephora terrestris*. The identity of the different mycorrhizae is discussed later. This competition with *T. terrestris* for feeder roots may have accounted for the lack of dominance of *P. tinctorius* on the seedling roots. Competition between these fungi was observed recently on container-grown seedlings of *P. taeda.* Seedlings were inoculated at two, four, six, and eight weeks after germination with basidiospores of *P. tinctorius* (Ruehle 1980). The older seedlings which already had a few *T. terrestris* mycorrhizae at inoculation formed fewer mycorrhizae with *P. tinctorius* in the same period of time than younger seedlings inoculated with *P. tinctorius* before *T. terrestris* could colonize a substantial part of their root systems.

In these and subsequent experiments carried out by Institute scientists, the degree of ectomycorrhizal development is expressed as a percentage of all the short roots infected. Normally the introduced fungus occurs in mixtures on the roots with naturally occurring fungi. Therefore, the amount of mycorrhizae formed by the introduced fungi is expressed as a part of the total percentage of mycorrhizae formed.

Basidiospores of *P. tinctorius* have also been used in conventional nurseries in the southern United States. Following effective soil fumigation in two different tree nurseries, ectomycorrhizae were formed with basidiospores on seedlings of *Pinus taeda, P. elliottii* var. *elliottii, P. virginiana, P. clausa,* and *P. strobus* after one growing season. The freshly collected spores were incorporated into the soil at a rate of $2.55 \times 10^9/m^2$ of soil surface just prior to seeding. The success of soil infestation with the basidiospores varied. On *P. clausa* in Florida, *Pisolithus* accounted for about 12 per cent of all the ectomycorrhizae, and on *P. taeda* and *P. strobus* in North Carolina it accounted for nearly 70 per cent of all the ectomycorrhizae. Naturally occurring fungi formed the remaining mycorrhizae. *Pisolithus tinctorius* failed to dominate the root systems. More basic studies on *P. tinctorius* basidiospores (Marx 1976) revealed that even in a soil environment free of competing ectomycorrhizal fungi, it takes basidiospores at least two months after seed germination to form macroscopically detectable ectomycorrhizae and four months to stimulate growth of *P. taeda* seedlings. During this period other fungi obviously can

colonize roots in a natural soil. These studies also revealed that basidiospores collected from dry, insect-free sporophores can be stored in amber bottles at 5 °C for 34 months without loss of capacity to synthesize mycorrhizae (Marx 1976). Currently these spores are used for ectomycorrhizal synthesis; they have been stored for over five years under these conditions.

In the spring of 1975 basidiospores of *P. tinctorius*, as well as mycelial inocula of *P. tinctorius* and other fungi, were successfully used to correct the erratic occurrence of ectomycorrhizal fungi in a new tree nursery in south-eastern Oklahoma (Marx *et al.* 1978). Basidiospores were added to fumigated and non-fumigated soil just prior to seeding at rates of 1.19, 3.56, and 7.13 × 10^9 basidiospores per m^2 of soil surface. Seedlings of *P. taeda* formed abundant *Pisolithus* mycorrhizae in all plots after one growing season. There were, however, no well-defined differences in the amount of *Pisolithus* mycorrhizae formed in plots initially infested with different quantities of basidiospores. Basidiospores formed about 50 per cent more ectomycorrhizae on seedlings in fumigated soil than in non-fumigated soil. In fumigated soil, 70 per cent of all the mycorrhizae on seedlings were formed by *P. tinctorius*, whereas in non-fumigated soil *Pisolithus* accounted for less than half of all the mycorrhizae. Other ectomycorrhizae were formed by naturally occurring fungi. Fumigation eradicated these latter fungi and other microbial competitors increasing the effectiveness of the *Pisolithus* basidiospores.

Another study was installed in the same nursery in the spring of 1976 to examine different practical methods of infesting soil with basidiospores of *P. tinctorius* (Marx, Mexal, and Morris 1979). Basidiospores (stored at 5 °C for eight months) were added to fumigated soil prior to seeding by (a) mixing spores in a hydromulch (wood pulp suspended in water) and broadcasting with a tractor-drawn applicator, (b) dusting spores onto the soil surface, or (c) injecting spores into soil with a tractor-mounted injector. Two other treatments were (d) dusting spores or (e) drenching spores onto seedlings six weeks after seeding. The rate of basidiospore application in all treatments was 5.5 × 10^8 per m^2 of soil surface. After one growing season the 350 000 seedlings of *P. taeda* were lifted and evaluated. Spores mixed with the hydromulch (a) were the most effective treatment. Three-quarters of the seedlings in this treatment had *Pisolithus* mycorrhizae and these represented over one-quarter of the total formed on the seedlings. This development resulted in a 15 per cent increase in the number of plantable seedlings and stimulated overall seedling growth (fresh weight) by 25 per cent over non-inoculated controls. The next best treatment was (b), dusting spores onto the soil at time of seeding. Only one-third of the seedlings

this treatment had *Pisolithus* mycorrhizae, and these only represented about one-tenth of all the mycorrhizae on the seedlings. There were 13 per cent more plantable seedlings in this treatment and seedling fresh weights were approximately 12 per cent greater than the controls. There are problems in using dry basidiospores. During dusting the dry spores are difficult to control because breezes carry them great distances from the intended plot. This inconsistency of soil inoculation caused erratic development of mycorrhizae. All other methods of spore inoculation were not very effective. *Thelephora terrestris* formed abundant ectomycorrhizae on all seedlings in this study. Basidiospores of this fungus came from the numerous sporophores produced under pines planted adjacent to the nursery a few years earlier to provide a natural inoculum source (Marx *et al.* 1978).

Container-grown pine seedlings have been inoculated with basidiospores of *P. tinctorius* (Marx and Barnett 1974; Ruehle and Marx 1977). Root substrates such as vermiculite, peat moss, and pine bark, used in containers in the United States are successfully inoculated with mycorrhizal fungi because these substrates normally contain few microbial competitors. In greenhouse studies, equal success is achieved in forming *Pisolithus* mycorrhizae by dusting basidiospores onto seedlings (in a wind-free area) or mixing spores directly into the root substrate prior to seeding. Another promising technique is to mix basidiospores of *P. tinctorius* in the external matrix of encapsulated pine seed. For the past year the forest division of Hilleshög Seed Company Ltd., Landskrona, Sweden, and our research group in Athens, Georgia have been working co-operatively on the development of this technique. Encapsulation permits many spores to be placed on individual seed. However, the encapsulating material must be non-toxic to the spores and to the seed, and it must degrade rapidly after planting to permit satisfactory spore release onto the root zone.

It is obvious that spores of ectomycorrhizal fungi can be used in a variety of ways to either infest soil or inoculate seed for mycorrhizal development on seedlings in nurseries and containers. Results are not always positive, however. During the past eight years (Marx, unpublished data) basidiospores of a variety of fungi have been carefully collected, stored briefly, and used to infest steamed or fumigated soil in a special mycorrhizal fungus-free growth room in Athens (Marx 1973). With the exception of *P. tinctorius* and *T. terrestris*, basidiospores of *Amanita muscaria, A. caesarea, A. rubescens, Paxillus involutus, Lactarius deliciosus, L. piperatus, L. indigo, Laccaria laccata, Suillus luteus, Clitocybe nuda,* and *Russula emetica* did not form mycorrhizae on *Pinus taeda* or *P. echinata* seedlings in a four- to six-month test period. Trappe (1977) has also encountered difficulties

in forming mycorrhizae on western conifers with basidiospore inoculum of various fungi collected in the Pacific Northwest of the United States, as has Shemakhanova (1962) in Russia with various tests on oak. Obviously, a great deal more research is needed on collecting, storing, handling, and inoculating procedures for spores of ectomycorrhizal fungi before they can be successfully used in inoculation programmes.

Advantages and disadvantages

There are advantages and, unfortunately, certain disadvantages in using spores of ectomycorrhizal fungi for inoculation purposes. The major advantage is that they require no extended growth phase under aseptic conditions in the laboratory as does the production of vegetative mycelial inoculum (see later discussion). Another advantage is their lack of bulk. According to Donald (1975), there are approximately 11 million spores per gram of ground sporophores of *Rhizopogon luteolus*. Marx and Bryan (1975) report approximately 1.1×10^9 spores of *P. tinctorius* per gram of basidiospores. Large numbers of basidiospores can be collected from mature sporophores of many ectomycorrhizal Gasteromycetes such as *Pisolithus*, *Rhizopogon*, and *Scleroderma*. In less than 12 man hours, we have extracted 12 kilograms of basidiospores of *P. tinctorius* from sporophores collected from under young loblolly pines growing on a kaolin spoil in central Georgia. This one collection contained 12.5×10^{12} basidiospores. If these spores were used at a rate of 5.5×10^8 spores/m^2 of soil surface, this collection could be used to inoculate 5.5 million seedlings. It would be nearly impossible to collect this quantity of spores from any of the other ectomycorrhizal fungi, especially those belonging to the Agaricales or Aphyllophorales, but rapid collection is an advantage of *Pisolithus*. Another advantage of spores, at least those of certain fungi, is that they can be stored from one season to the next. This is important since spores collected in the summer or early autumn would normally have to be stored until the following spring if they are to be used to inoculate nursery seedlings.

There are also certain disadvantages in the use of spores as inoculum. Spores of many fungal species cannot be germinated to determine their viability. Hile and Hennen (1969) reported low germination of basidiospores of *P. tinctorius* on agar plates and were unable to make successful single spore transfers to new media. Lamb and Richards (1974c) tested different conditions of pH, temperature, and relative humidity and found that under the best test conditions only 0.38 per cent of the basidiospores of *P. tinctorius* would germinate. Basidiospores of other fungi, however, germinated much better than those of *P. tinctorius*. For years (Marx, unpublished data) various

physical and chemical stimuli were used to germinate basidiospores of *T. terrestris* and *P. tinctorius* without success. Apparently, synthesis of mycorrhizae is the only reliable means to determine viability of different spore collections of *P. tinctorius*. However, precise quantification of viable spores is difficult using the synthesis procedure (Marx 1976).

Other disadvantages are that the quantity of sporophores of many fungi required to inoculate nurseries may not be available every year and spore collections are frequently contaminated with various microorganisms. This is especially true of collections from Gastromycetes such as *P. tinctorius* where the basidiospores are exposed to the elements for several days or weeks during their maturation. Although data are not available, these contaminants may affect the health of tree seedlings or viability of spores.

The biggest disadvantage of using spores to inoculate seedlings is that it takes them several weeks to form mycorrhizae. This infection process is much slower than that achieved with mycelial inoculum (Marx, Bryan, and Cordell 1976; Theodorou and Bowen 1970). During this period of ingress less desirable fungi, such as pathogens (Marx 1972) or other ectomycorrhizal fungi, can colonize the roots and reduce the effectiveness of the introduced spore inocula. However, in parts of the world where the occurrence of ectomycorrhizal fungi is erratic or deficient, this delay may not have a significant effect on the final amount of mycorrhizae developed on tree seedlings from spore inoculum.

Mycelial inoculum

Ectomycorrhizal fungi as a group are difficult to grow in the laboratory. Many have never been isolated and grown in pure culture. Some species that have been isolated grow slowly, others often die after a few months in culture. Most ectomycorrhizal fungi require specific growth substances, such as thiamine, biotin, and simple carbohydrates, and are very sensitive to growth inhibiting substances (Palmer 1971).

The use of pure mycelial cultures of ectomycorrhizal fungi has been repeatedly recommended (Bowen 1965; Marx 1977a; Mikola 1973; Shemakhanova 1962; Trappe 1977) as the most biologically sound method of inoculation. Unfortunately, large scale nursery application of pure mycelial cultures has been severely hampered by the lack of sufficient amounts of inoculum. It may be possible to produce sufficient inoculum for research studies in small containers, pots, microplots, or even small nursery plots, but it is something else to produce a sufficient quantity of mycelial inoculum of an ectomycorrhizal fungus for a large nursery.

Another problem with pure mycelial cultures is knowing which fungal species to use under different conditions or with different hosts. During the past two decades a great deal of data on differences between mycorrhizal fungi and their differential effects on trees has been published (Bowen and Theodorou 1973; Marx 1977a; Theodorou and Bowen 1970). The first step in any nursery inoculation programme, therefore, must be the careful selection of suitable fungi (Bowen and Theodorou 1973; Mikola 1973; Trappe 1977).

Several researchers in various parts of the world have developed cultural procedures for producing pure mycelial inoculum of a variety of fungi for research purposes. In the last couple of decades, some of these procedures have been extensively used for various small experiments. Published information is available from Austria, Argentina, Australia, and, more recently, the United States. Experiments with pure culture inoculations have also been conducted in the Soviet Union, but details of these procedures or results have not been described (Levisohn 1958; Lobanow 1953; Mikola 1973). According to Wilde (1971), the use of pure cultures in the Socialist Republics in Europe have failed to produce significant results due to indigenous ectomycorrhizal fungi distributed throughout the soils. There are also numerous experiments with inconsistent or negative results. Since scientists tend to publish only positive results, experimental failures probably occur more frequently than we know from reviewing the literature (Mikola 1973).

Austria

Techniques used in Austria are based primarily on the work of Moser (1958a,b,c,d, 1959, 1961, 1963, 1965). Apparently techniques were developed initially to inoculate seedlings of *Pinus cembra* with low temperature strains of *Suillus plorans* in the nursery. This fungus was absent from the warmer nursery soils in the valley and in the alpine meadows, but it is a highly desirable fungal symbiont for the reforestation of this pine on the cold, high elevation sites near the timberline. Reforestation of these high elevation, mountainous areas is desirable in order to prevent avalanches and landslides.

For production of inoculum, *Suillus plorans* is first grown on Moser's (1958b) nutrient solution in small flasks for several days. The mycelium is transferred to 10-litre tanks containing the same nutrient solution and aerated for two to three hours daily for three to four months. The mycelium and liquid are poured into 5-litre flasks containing sterilized peat moss and fresh nutrient solutions. During the next few months, *S. plorans* grows throughout the substrate; the inoculum is then ready for use. Although attempts are made to maintain these cultures in aseptic condition, contaminations

by *Penicillium*, *Mucor*, and bacteria often occur. Moser (1963) refers to this contaminated inoculum as 'half-pure cultures' and claims that on certain occasions it proves more effective in forming mycorrhizae than pure cultures. He speculates that these contaminants add a 'rhizosphere effect' to the inoculum which is beneficial to ecto-mycorrhizal development and seedling growth. He also found that the most effective inoculum of *S. plorans* and other fungi is produced in organic materials such as sterile forest litter, ground peat, or saw-dust. He observed best results with ground peat. Very inconsistent results were observed with agar inoculum or mycelial suspensions. Other workers (Ekwebelam 1973; Levisohn 1956; Mikola 1973; Marx, unpublished data) have used agar inoculum or mycelial suspensions with varying degrees of success.

Inoculum removed from the culture tanks is packaged in sterile polyethylene bags, transported to the nursery, and when possible applied to nursery soil within three days. The inoculum is usually placed in 10 cm deep furrows in the soil at a rate of 3 to 4 litres of inoculum per m^2 of soil surface. Young (one-month-old) seedlings of *P. cembra* are then transplanted into these furrows. The best mycorrhizal development occurs on seedlings growing in soil previously sterilized with heat or formalin.

Moser (1959) reported other means of using pure mycelial inoculum. It can be broadcast 1 cm deep onto soil and then chopped 10 cm deep into the soil prior to seeding. This method requires much larger amounts of inoculum (8–10 l/m^2 of soil surface) and the inoculum often dehydrates on the soil surface prior to its incorporation. With this method the inoculum must be able to survive in soil for the elapsed time between seeding and when short roots are formed on seedlings. With certain tree species in Austria, ectomycorrhizae may not form for eight months following soil infestation and seeding. This means that inoculum added to soil at sowing must be able to survive a rather long period in the absence of a host. Transplanting seedlings into furrows containing inoculum is preferred because it eliminates this problem. Since transplanted seedlings of certain tree species must remain in the nursery for two years (*P. cembra* is grown in the nursery for up to four years), there is the option of only inoculating every third or fourth row of seedlings. According to Moser (1963), once root infection has taken place the introduced fungi spread to adjacent seedlings. Another method of soil inoculation is placing inoculum in furrows between rows of established seedlings. This method is successful, but not recommended because digging furrows near seedlings can damage roots.

Moser (1963) has also used this technique to produce mycelial inoculum of *Suillus placidus*, *S. grevillei*, *S. aeruginascens*, *Paxillus*

involutus, Amanita muscaria, and *Lactarius porninsis*, either alone or in mixtures. Although Moser only presented limited quantitative data from different fungi/tree species tests in nurseries (1958a,b,c,d, 1959, 1961, 1963, 1965), the results show the biological significance of the inoculation. In one of Moser's nursery tests in a sandy alluvial soil, pure mycelial inoculum of *Phlegmacium glaucopus* formed abundant ectomycorrhizae on spruce. The inoculated seedlings had a healthy green colour and were considerably larger than non-inoculated seedlings, which had chlorotic foliage and roots completely free of ectomycorrhizae. Moser failed to mention the form of inoculum used, the method of inoculation, or the duration of the nursery test. In other tests (Moser 1963), larch seedlings were grown in both sterilized and non-sterilized soil of different types inoculated with *S. grevillei, S. aeruginascens, L. porninsis,* and a mixture of the three fungi. A fifth treatment was a control without inoculation. After two years the non-inoculated seedlings in the non-sterile soil from a spruce forest had an ectomycorrhizal frequency of 56, which was similar to that of seedlings from non-sterile soil inoculated with the fungi. The mycorrhizal frequency of the fungal treatments varied from 53 to 72. However, the non-inoculated seedlings in sterilized soil did not form ectomycorrhizae, while those in the fungal treatments had mycorrhizal frequency rates of 18 to 79. These data proved the importance of soil sterilization as a prerequisite to the effective use of mycelial cultures. Although no mention was made of the type of soil sterilization used, it obviously was successful in eliminating the indigenous symbiotic fungi. In a similar test using soil from a meadow, Moser (1963) found a much lower mycorrhizal frequency on inoculated seedlings from the same fungal treatments, especially on those growing in non-sterilized soil. The meadow soil not only had fewer indigenous symbiotic fungi, but also had a reduced potential for mycorrhizal development following inoculations with the fungi. Moser (1959, 1963) discussed other nursery experiments but did not provide data on seedling growth or mycorrhizal development.

Recently in Austria, Göbl (1975), a co-worker of Moser, discussed the selection and culture of specific ectomycorrhizal fungi for nursery inoculations and methods of producing sufficient amounts of inoculum for practical use. She generally follows the procedures of Moser and recommends growing the fungi in a liquid medium until adequate mycelium is obtained. This mycelium is placed in 1-litre bottles containing cooked and sterilized cereal grains such as wheat or white millet. Calcium sulphate (0.4 to 0.5 g/100 g of grain) is added to improve the growth of certain fungi. These grain cultures are shaken lightly each week and after two to four weeks at 20 to 22 °C

the grains become thoroughly colonized by the fungi. The grain cultures can then be stored at 4 to 6 °C for up to nine months. Göbl recommends that the grain culture be checked periodically for microbial purity on an appropriate agar medium.

The grain cultures are added to enriched peat moss for the final stage in the production of inoculum. The peat must be enriched with nitrogen and carbohydrates (ammonium tartrate, asparagine, soyabean meal, blood meal, malt extract, glucose), as well as inorganic nutrients in different combinations. The kinds and amounts of these supplements vary according to the species of fungus grown. Usually 7 to 10 grams of glucose per litre of peat is used as a standard for carbohydrates. Ten to 15 litres of sterile, enriched peat moss is placed in large transparent plastic bags and inoculated with a generous supply of grain culture. The plastic bags are plugged with cotton to provide aeration and are shaken occasionally during storage at 20 to 22 °C. After three to six weeks the inoculum is ready for use in the nursery. Contaminated cultures are apparently discarded. This method has been used to produce inoculum of *Suillus plorans, S. grevillei, Boletinus cavipes, Amanita muscaria,* and *Hebeloma crustuliniforme.* An interesting idea presented by Göbl (1975) was that the last phase involving the sterile peat moss could be done at the nursery. The problems created by shipping large volumes of peat moss inoculum would be eliminated by shipping just the grain cultures to the nursery.

After satisfactory inoculum has been produced it can be used to infest soil according to the various procedures of Moser (1963). Göbl (1975) recommended another unique method, which is to transplant a young tree seedling directly into inoculum contained in a larger volume of peat moss. After the peat moss supporting the seedling becomes colonized by the introduced fungus it is used for inoculum. Göbl (1975) prefers this form of inoculum to forest litter because it eliminates the introduction of unknown microbial populations into the nursery.

Argentina

Techniques used in Argentina were developed by Takacs (1961, 1964, 1967) at the Mycorrhiza Laboratory of the Instituto Nacional de Tecnologia Agropecuaria (INTA) at Castelar. When new pine nurseries are established in formerly treeless areas lacking ectomycorrhizal fungi the soil is inoculated with pure mycelial cultures. Techniques are very similar to those developed by Moser in Austria. Basidiospores or pieces of tissue from the sporophores are cultured on an appropriate agar medium. The mycelium is transferred to liquid culture, incubated, and added either to sterilized, germinated grains of cereals

(such as barley), the cereal chaff, a mixture of grain and chaff, or sterilized peat moss. All substrates are enriched with a liquid medium. Takacs (1967) inoculated the substrates either with mycelial agar discs or mycelium from liquid culture. This inoculum, regardless of the physical media, is used after one to two months' incubation at room temperature. Peat moss is used more commonly than the other substrates. Pure mycelial cultures of *Amanita verna, Suillus granulatus, S. luteus, Hebeloma crustuliniforme,* a *Russula* sp, *Scleroderma verrucosum,* and *S. vulgare* have been produced by this method and are apparently available from the INTA in Argentina (1967). The details for large scale nursery inoculation in Argentina using Takacs's method were described by Mikola (1969). Usually five 200-ml flasks of each of four different fungi contained in either peat moss or grain-chaff inoculum are sent from INTA to a nursery. Upon arrival at the nursery the contents of each of these 20 flasks are mixed with 4 to 10 kg of sterilized soil or forest litter. These mixtures are kept moist and incubated for three weeks before use in the nursery beds. Using this method, twenty 200 ml 'starter' cultures can be used to produce 100 to 200 kg of soil inoculum. According to Mikola (1969), this is sufficient to infest 500 m^2 of nursery soil. Inoculum is usually added to the soil during preparation of the nursery beds.

Since quantitative data are not available for this work it is difficult to evaluate the success of this method on a large nursery scale. However, based on our current knowledge it is difficult to believe that these fungi can grow saprophytically in the sterile soil or litter at the nursery. Mycelial growth must occur in the presence of competitive micro-organisms and in the absence of essential nutrients. If the starter inoculum survives the incubation in soil or litter at the nursery, perhaps all that is really accomplished is a dilution of the original inoculum. This diluted inoculum must be sufficient to effectively colonize seedling roots in these nurseries containing few, if any, ecto-mycorrhizal fungi which can compete with the introduced inoculum.

There is only limited quantitative data available to this author on the earlier experimental work in Argentina preceding the broad application of mycelial inoculations. In one test inoculation was done with mycelial discs from Petri dish culture and not with inoculum prepared by any of the previously mentioned methods. Takacs (1964) isolated *Scleroderma vulgare* from sporophores collected from a plantation of *Pinus taeda* and grew it on an agar medium. A nursery soil was mixed 4:1 with sand, sterilized with methyl bromide and 10 per cent formalin, and planted with seed of *P. taeda.* Sixty days after seed germination half the seedlings were inoculated with pieces of agar containing the fungus. These mycelial pieces were placed 10 cm apart and 3 to 4 cm deep in the soil. When all the seedlings were

lifted and measured after ten months, the results proved the value of inoculation. Inoculated seedlings formed abundant ectomycorrhizae and the soil from which they were lifted was a grey colour, apparently caused by the grey mycelium of *S. vulgare* colonizing the soil. Total fresh weights of the inoculated seedlings were 83 per cent greater than the non-inoculated controls. The differences were highly significant based on statistical analysis. Although it was not mentioned, it is assumed that seedlings in non-inoculated soil were free of mycorrhizae.

In an earlier test, Takacs (1961) used grain cultures of different fungi. Pure cultures of *Suillus granulatus*, *Scleroderma vulgare*, *Amanita phalloides*, and a *Russula* sp were obtained by germinating the spores on a liquid medium. The mycelium was used to inoculate sterilized, germinated barley seed. After only five days of incubation the grain cultures, according to Takacs, were ready to be used as inoculum. *Pinus pinaster*, *P. radiata*, and *P. thunbergii* were seeded in non-sterilized soil in a nursery bed. After 30 days, one or two grains of barley colonized by the specific fungi were placed 20 cm apart in the row next to the seedlings roots. Grain cultures of the four fungi were placed alternately in the rows. Apparently the seedlings were harvested several months later in the autumn. No quantitative data on mycorrhizal development was presented in this report but numerous ectomycorrhizae were illustrated. Takacs (1961) simply stated that the inoculated seedlings had exceptional growth and were considerably larger than the non-inoculated seedlings. No mention was made of other ectomycorrhizal fungi. It is difficult for this author to understand how these fungi spread rapidly enough from grain cultures placed so far apart to produce mycorrhiza on all seedlings. Another test was installed the following spring in different nurseries using the same techniques. Grain cultures of these four fungi were mixed together into non-sterile soil of three nurseries just prior to seeding. Germination began normally, but in two nurseries damping-off destroyed nearly 50 per cent of the seedlings in inoculated plots. The grain cultures probably contributed to the development of the damping-off micro-organisms. The seedlings were evaluated after six months. Takacs stated that the larger green seedlings had abundant ectomycorrhizae and the smaller chlorotic seedlings lacked mycorrhizae. However, he failed to report whether the large seedlings with ectomycorrhizae came from inoculated plots.

Australia

Theodorou (1967) developed pure mycelial inoculum of *Rhizopogon luteolus* using techniques similar to those of Moser. The purpose of inoculation with *R. luteolus* was to correct the deficiency

of ectomycorrhizal fungi in some Australian soils and also to produce seedlings from *Pinus radiata* with a root system having a greater capacity to absorb phosphorus from soil. Earlier work by Bowen (1962) showed that *P. radiata* seedlings had a better uptake of phosphorus with mycorrhizae formed by *R. luteolus* than with other fungi. Pure cultures were produced in a medium of vermiculite, chaff, and corn meal in a ratio of 10:2:1 moistened with a liquid medium. The fungus was placed in bottles containing about 80 grams of medium and incubated for one month at 25 °C. Twenty-five grams of inoculum were buried 8 cm deep in soil contained in pots which had either been steamed, fumigated with different rates of methyl bromide, or non-sterilized. Certain pots were reinoculated with 10 grams of non-sterile soil. All pots were seeded with *P. radiata* and seedlings were evaluated after nine months. Since Theodorou (1967) did not use non-inoculated, sterilized soil as a control, we must assume that all ecto-mycorrhizae were formed by the introduced fungi and not from naturally occurring fungi. In steamed or methyl bromide sterilized soil that was not reinoculated with non-sterile soil, mycorrhizal development varied from 33 to 41 per cent. Ectomycorrhizal development varied from 17 to 31 per cent in sterilized soil containing *R. luteolus* as well as the non-sterile soil. Substantial increases in growth of *P. radiata* seedlings were correlated with mycorrhizal development. Best growth occurred in sterilized soil containing only inoculum of *R. luteolus*. Theodorou concluded that the effectiveness of *R. luteolus* mycelial inoculum is suppressed by antagonistic soil organisms and, therefore, recommends sterilization of soil prior to artificial inoculation with this fungus. In another greenhouse study, Theodorou and Bowen grew freshly collected cultures of *Suillus granulatus, S. luteus, Cenococcum graniforme,* and *Rhizopogon luteolus* in vermiculite medium for three weeks as described above. The inoculum was mixed into the upper 8 cm of steamed soil contained in pots. A non-inoculated, sterile soil control was used. All pots were seeded with *P. radiata*. The study was terminated after 14 months and seedlings evaluated. Ectomycorrhizal assessments were done both macro- and micro-scopically. The degree of mycorrhizal development on seedlings inoculated with *R. luteolus, S. granulatus, S. luteus, C. graniforme,* and the controls was 20, 12, 16, 2, and 6 per cent, respectively. Dry weights of seedlings with *R. luteolus* were 90 per cent greater and those with *S. granulatus* were 30 per cent greater than the other three seedling groups. *Suillus luteus* and *C. graniforme* mycorrhizae did not stimulate seedling growth. Seedlings with *Rhizopogon* mycorrhizae contained 47 to 125 per cent more phosphorus in foliage than control seedlings or those with other fungi, showing the ability of mycorrhizae formed by this fungus to enhance phosphorus absorption in *P. radiata*.

In another test, Theodorou and Bowen (1970) produced inoculum of fresh cultures of *S. granulatus*, *S. luteus*, and four isolates of *R. luteolus* and inoculated soil sterilized by gamma irradiation. The pots were seeded to *P. radiata* and seedlings evaluated after two years. Although quantitative data on ectomycorrhizal development was not presented, the authors stated that all inoculated seedlings had very good ectomycorrhizal development and non-inoculated seedlings lacked mycorrhizae. Over 100 per cent differences in dry weights of seedlings were obtained between fungi. The growth of all inoculated seedlings was significantly better than the controls. There was as much as 85 per cent difference in dry weight of seedlings induced by different isolates of *R. luteolus*. As a group the *R. luteolus* isolates were superior to the other fungi in stimulating seedling growth.

United States

Tests to artificially introduce pure mycelial cultures of ectomycorrhizal fungi into soil were begun in the early 1930s by Hatch (1936, 1937). He grew seedlings of *Pinus strobus* in non-sterile prairie soil in large pots. These pots were housed in a chamber filtered to exclude contamination from air-borne spores of mycorrhizal fungi. Three months after seeding the seedlings were small, yellow, unthrifty in appearance, and devoid of mycorrhizae. Half of the seedlings were inoculated with agar cultures of *Suillus luteus*, *Boletinus pictus*, *Lactarius deliciosus*, *L. indigo*, and *Cenococcum graniforme*. After five months, root evaluations revealed that *S. luteus* and *L. deliciosus* formed mycorrhizae on 30 per cent of the short roots and stimulated seedling growth. The other fungi apparently failed to form mycorrhizae. Non-inoculated seedlings remained stunted and chlorotic. Hatch proved that pure cultures of specific fungi could be used to correct the deficiency of mycorrhizae. Once the natural soil lacking mycorrhizal fungi was inoculated and mycorrhizae were formed, it supported normal growth of white pine seedlings.

Three decades passed before Hacskaylo and Vozzo (1967) initiated a series of inoculation experiments in Puerto Rico with pure mycelial cultures of various fungi. In one test (Vozzo and Hacskaylo 1971) pure mycelial inocula of *Cenococcum graniforme*, *Corticium bicolor*, *Rhizopogon roseolus*, and *Suillus cothurnatus* were used. These fungi were selected because they were proven symbionts and had distinctive hyphal colours which should aid in subsequent evaluations. Following Moser's (1963) general technique, they grew the fungi on agar and then in liquid media. The mycelium from liquid culture was used to inoculate polypropylene cups containing a 2:1 ratio of sterile peat moss and vermiculite moistened with a glucose–ammonium tartrate nutrient solution (pH 3.8). After 16 weeks of incubation the inoculum

was flown from the USDA, Forest Service, Pioneering Research Unit Laboratory in Beltsville, Maryland, to Puerto Rico. In Puerto Rico, seedlings of *Pinus caribaea* were grown in a non-mycorrhizal condition for four months in a container nursery where they were watered and lightly fertilized. Seedlings were grown in 8 X 15 cm plastic bags filled with a 1:1 mixture of fumigated peat moss and vermiculite. The plastic bags were split and one-half cup of inoculum was placed against the exposed non-mycorrhizal roots of each seedling. The bag was closed and slipped into another container to hold the inoculum and root substrate intact. Ten months later the seedlings were measured and evaluated for mycorrhizal development. Certain seedlings were outplanted in the field; these results will be discussed later. Although they did not present quantitative data on mycorrhizal development, Vozzo and Hacskaylo (1971) reported that *Corticium bicolor, Rhizopogon roseolus,* and *Suillus cothurnatus* formed mycorrhizae. *Cenococcum graniforme* did not form mycorrhizae. Seedling height growth was correlated with ectomycorrhizal development, i.e. seedlings with the most ectomycorrhizae (*Corticium bicolor*) were the tallest. This study and others in Puerto Rico were complicated by the frequent occurrence of *Thelephora terrestris* sporophores and mycorrhizae throughout the nursery and on test seedlings. This fungus was introduced from the United States in pine duff inoculum in 1955 to correct the chronic deficiency of mycorrhizal fungi in Puerto Rico (Briscoe 1959).

Mycorrhizal Institute

In the south-eastern United States, formal research on the use of pure mycelial cultures began in 1966 at the USDA, Forest Service Laboratory in Athens, Georgia. In 1976, the research unit was given greater research latitude and was designated the Institute for Mycorrhizal Research and Development. One of several research goals of this multidisciplinary unit is to perfect existing techniques and to devise new ones for artificially inoculating tree seedlings with pure cultures of ectomycorrhizal fungi in bare-root and container nurseries. It is anticipated that these techniques will be valuable for not only correcting the erratic occurrence of ectomycorrhizal fungi in nurseries, but show the biological feasibility and practical value of manipulating and managing specific, highly desirable, ectomycorrhizal fungi on tree seedlings to improve the survival and growth of seedlings on routine and adverse reforestation sites.

Selecting fungi.   Initial research efforts were concentrated on *Pisolithus tinctorius, Thelephora terrestris, Cenococcum graniforme*, and a few other fungal species. *Pisolithus tinctorius* was chosen because it

is readily propagated in the laboratory on a variety of agar or liquid media. It had yellow-gold hyphae and mycorrhizae which aid in its detection and quantitative assessment on seedling roots. The main reason for selecting this symbiont, however, was its apparent ecological adaptation to adverse soil conditions such as those found on coal spoils (Marx 1977a; Schramm 1966). *Pisolithus tinctorius* is also widespread on trees growing on kaolin spoils, sheet-eroded soils, borrow pits, and other biologically hostile sites. These sites are characterized by one or more adverse soil conditions—high soil temperatures, extreme acidity, high levels of Al, Mn, S, Fe, and chronic low fertility—which limit routine reforestation.

Seedlings with *Pisolithus* mycorrhizae formed in the nursery prior to outplanting on adverse sites should survive and grow better than routine nursery seedlings having *Thelephora* or other mycorrhizae. Tree seedlings 'tailored' with *P. tinctorius* should have a physiologically and ecologically adapted root system capable of surviving and persisting in adverse soils. The concept of forming mycorrhizae on seedlings with fungi ecologically adapted to the planting site parallels that proposed by Moser (1963) who used mycorrhizae formed by *Suillus plorans*, a low temperature fungus, on *Pinus cembra* to enhance reforestation of the high, cold, elevation sites in Austria.

There are results from some basic studies which help explain the persistence of *P. tinctorius* on these sites. In controlled temperature studies (Marx, Bryan, and Davey 1970) it was found that *P. tinctorius* was tolerant of high temperatures. The fungus grew in agar culture over a temperature range of 7 to 40 °C with an optimum at 28 °C. Later Momoh and Gbadegesin (1975), using a Georgia isolate of *P. tinctorius*, successfully grew mycelium of the fungus at 42 °C with an optimum at 30 °C. Lamb and Richards (1971) reported that the thermal death point for hyphae of *P. tinctorius* was 45 °C, whereas hyphae of *Rhizopogon luteolus* and other fungi were killed at 38 °C. Marx, Bryan, and Davey (1970) also reported that *P. tinctorius* formed more ectomycorrhizae on seedlings of *Pinus taeda* grown in aseptic culture at 34 °C than at lower temperatures. Later Marx and Bryan (1971) produced aseptic seedlings of *P. taeda* with different ectomycorrhizae at 25 °C and exposed them to higher temperatures. Seedlings with *Pisolithus* mycorrhiza had better survival and growth at 40 °C than non-mycorrhizal seedlings or seedlings with *Thelephora* mycorrhiza. *Pisolithus tinctorius* is also tolerant of and can be stimulated by the heavy metals often found in adverse sites such as coal spoils. Hile and Hennen (1969) found that the addition of iron and sulphur to agar medium stimulated vegetative growth of *P. tinctorius*. Muncie, Rothwell, and Kessell (1975) detected large amounts of elemental sulphur in the interior of sporophores of the fungus

collected from coal spoils. These authors suggested that *P. tinctorius* has a unique, but unexplained, method of utilizing sulphur. These various studies showing the high temperature and metal tolerance of *P. tinctorius* help explain its ability to survive on coal spoils and other adverse sites.

There are several other unique features of *P. tinctorius* which make it a potentially strong fungal candidate for practical use in a variety of different forest situations. In addition to its occurrence on adverse sites, it also occurs in urban areas, orchards, and routine forest sites. It has been reported to occur in tree nurseries (Marx 1977b), especially on two- to three-year-old pine seedlings in northern nurseries of the United States (Marx, unpublished data). *Pisolithus tinctorius* has a proven tree host-range of nearly 50 species and is associated with an additional 25 tree species. These trees include most of the world's more important species. This fungus occurs in 33 countries of the world and in 38 states in the United States (Grand 1976; Marx 1977b). Problems with plant quarantine regulations regarding the use of *P. tinctorius* in most parts of the world should be minimal because of its broad, natural geographic distribution. After considering all of these features it becomes apparent that the development of inoculum and inoculation techniques with this fungus might prove useful not only in reclamation of adverse sites, but in reforestation of routine forest sites with both indigenous and exotic tree species in various parts of the world.

Thelephora terrestris was selected for testing at the Mycorrhizal Institute because it also grows rapidly in the laboratory on a variety of agar and liquid media. Its hyphae and ectomycorrhizae on pine are white to cream-brown in colour (Marx and Bryan 1970; Marx, Bryan, and Grand 1970). Unfortunately this colour characteristic is common to a number of other ectomycorrhizal fungi and, therefore, visual detection and quantitative evaluation of *Thelephora* mycorrhizae are difficult. This fungus does, however, fruit on seedling stems or adjacent to seedlings on soil in nurseries and can be readily traced from sporophores to mycorrhizae via visible hyphal strands. This fungal symbiont is also widespread on tree seedlings in nurseries, as previously described, and has a broad host range (Hacskaylo 1965; Marx and Bryan 1969b, 1970; Weir 1921). Its common occurrence in nurseries suggests that it is ecologically adapted to the good tilth, fertility, and moisture conditions of nursery soils. Obviously *T. terrestris* is one of the major ectomycorrhizal fungi on roots of the enormous numbers of tree seedlings produced in nurseries and planted out annually on the millions of hectares of reforestation and reclamation sites all over the world. Its occurrence in nurseries also indicates that it is a major competitor to introduced inoculum of other fungi for seedling roots.

Cenococcum graniforme has been tested on a more limited scale at the Institute than the previously mentioned fungi. It was selected because of its easily identified, jet-black ectomycorrhizae, broad host-range (Trappe 1964), and apparent drought and high temperature tolerance (Mexal and Reid 1973; Meyer 1964; Saleh-Rastin 1976; Worley and Hacskaylo 1959). This last characteristic suggests that it may be valuable to seedlings planted on sites having seasonal drought conditions. Unfortunately vegetative growth of *C. graniforme* in pure culture is very slow. Using pure mycelial techniques, various researchers have also encountered difficulties forming mycorrhizae with this fungus on various tree species (Hatch 1936; Marx *et al.* 1978; Theodorou and Bowen 1970; Vozzo and Hacskaylo 1971).

Pure mycelial culture.   Our first attempts at producing pure mycelial cultures of *P. tinctorius*, *T. terrestris*, and *C. graniforme* were not very successful. None of these fungi would form mycorrhizae on seedlings of *Pinus taeda* from wheat grain cultures (Marx, unpublished data). Grain cultures were added to steamed soil in a 1:15 ratio in a mycorrhiza fungus-free growth room (Marx and Bryan 1969b; Marx 1973) and seeded to *Pinus taeda*. After five months none of the inoculated and control seedlings had mycorrhizae. Microscopic examination revealed that the grain cultures were colonized by saprophytic fungi and bacteria as early as three weeks after soil inoculation. We observed a great deal of damping-off of pine seed as did Takacs (1961). The high nutritive value of the boiled wheat contributed to its rapid colonization by saprophytes, which probably killed the ectomycorrhizal fungi. Our results do not support the claim of Park (1971) that grain cultures of *C. graniforme* can be used to inoculate nursery soil; our results from this and several other studies conflict with this broad recommendation. Our results with grain cultures also conflict with those of Takacs as discussed by Mikola (1973). Our grain cultures of the various fungi were decomposed so rapidly by saprophytic organisms that we doubt if grain cultures could be used at a nursery as a starter culture to inoculate sterile soil for the production of a large volume of inoculum.

Vermiculite and peat moss moistened with a modification of Melin-Norkrans medium (Marx 1969) with glucose instead of sucrose was found to be an excellent substrate for the production of mycelial cultures of these fungi. In one of our first tests (Marx and Bryan 1970), inoculum of *P. tinctorius* and *T. terrestris* was grown aseptically for four months at 25 °C in 1 litre volumes of a 28:1 ratio of vermiculite and peat moss moistened with nutrient solution. The inoculum was mixed in a ratio of 1:8 with an autoclaved mixture of soil:peat moss:vermiculite in the growth room. Seeds of various pine

species were planted, and after four months root evaluation revealed that *T. terrestris* formed mycorrhizae with 22 tree hosts and *P. tinctorius* with 14 tree hosts. All non-inoculated seedlings were free of mycorrhiza. This technique was successfully used later in the growth room in a study with *P. tinctorius* on *Pinus clausa* (Ross and Marx 1972), and again with *P. tinctorius* and *C. graniforme* on *P. echinata* (Marx 1973). In these and other studies, mycelium of *P. tinctorius* and *T. terrestris* usually completely permeated the vermiculite and peat moss particles after less than three months' incubation at 25 °C. However, because of the slow growth rate of *C. graniforme*, it is necessary to incubate this fungus for six to eight months under the same growing conditions to completely permeate the substrate.

Leaching of mycelial inoculum.   Our first tests outside the growth room using the vermiculite-peat moss inoculum in soil fumigated with methyl bromide gave variable results. After only a few weeks in the greenhouse the inoculum was extensively colonized by saprophytic fungi and bacteria in a fashion similar to that observed with the wheat grain cultures. This saprophytic colonization was markedly reduced by leaching the inoculum with water before adding it to soil. Leaching removed the non-assimilated nutrients and thus reduces the food base essential for saprophytic colonization by the micro-organisms. We compared several methods and types of liquids (physiological saline, sterile distilled water, and tap water) for leaching inoculum. The method which proved to be the best involved placing 4 litres of inoculum in a double layer of cheesecloth and irrigating this for several minutes under cool tap water. Excess water is removed by squeezing the inoculum in the cheesecloth by hand. This reduces the inoculum volume by one-third. In addition to non-assimilated nutrients, a great deal of pigment (a rich brown pigment in the case of *P. tinctorius*) and small vermiculite particles are washed from the inoculum during leaching. Other researchers (Moser 1963; Takacs 1967; Theodorou and Bowen 1970; Vozzo and Hacskaylo 1971) did not leach mycelial cultures prior to soil inoculation and certainly must have encountered competition problems with colonizing micro-organisms.

Leached vermiculite-peat moss inoculum of *P. tinctorius* was used successfully in 1972 to form mycorrhizae on pine at a microplot tree nursery (Marx and Bryan 1975). Inoculum was mixed at a ratio of 1:8 with fumigated soil contained in wooden framed microplots. Control soil was infested with autoclaved mycelial inoculum of *P. tinctorius* to standardize soil fertility and tilth. The soil was planted with seed of *P. taeda* in April and mulched. Periodic examinations of seedlings revealed that the mycelial inoculum of *P. tinctorius* was

effective in forming mycorrhizae on seedlings one month following seed germination. Numerous sporophores of *P. tinctorius* were produced in these plots during August to October. After the seedlings became dormant in December they were lifted and evaluated. The mycelial inoculum formed the distinctive gold-yellow ectomycorrhizae of *Pisolithus* on 92 per cent of the feeder roots which induced more than a 100 per cent increase in total dry weights of seedlings over the controls. The control seedlings had 45 per cent of their feeder roots colonized with the cream-brown ectomycorrhizae characteristic of *Thelephora terrestris*. The visual estimate of the amount of mycorrhizae formed by each of the fungi was confirmed by reisolation of the respective fungi from the mycorrhizae and also by a fluorescent antibody technique developed for each of the fungi (Schmidt, Biesbrock, Bohlool, and Marx 1974). From these experiments it was found that the mycorrhizae formed by these two fungi could be visually assessed accurately on intact seedlings with the unaided eye. It should be pointed out, however, that only one other ectomycorrhizal type was present on seedlings. It occurred infrequently and was a pure white, coralloid type, easily distinguished from either *P. tinctorius* or *T. terrestris* mycorrhiza.

Survival of mycelial inoculum.   A major concern in the use of pure mycelial cultures of ectomycorrhizal fungi expressed by other researchers (Moser 1963; Takacs 1967) is survival of inoculum in soil. Mycelial inoculum of *P. tinctorius* will survive in soil under a variety of conditions. During early months of the 1972 study (Marx and Bryan 1975), particles of the inoculum were removed periodically from the soil. Mycelium of *P. tinctorius* with good structural integrity was observed microscopically to be abundant in the laminated structure of the vermiculite particles. Mycelium within the leached vermiculite particles is apparently protected from environmental extremes and extensive saprophytic colonization. Residual inoculum will also survive in soil after overwintering. After the seedlings were removed from the microplots in December, soil which was initially infested with the mycelial cultures of *P. tinctorius* was left fallow until the following spring. Soil temperatures dropped to −7 °C on several occasions during the winter. In April soil in the plots was mixed with freshly fumigated soil at different ratios, seeded with *P. taeda*, and in December the seedlings were lifted and evaluated. Seedlings in non-diluted soil formed mycorrhizae with *P. tinctorius* on 75 per cent of the feeder roots. In the 1:1, 1:2.5, 1:5, and 1:10 dilutions, 60, 35, 25, and 15 per cent of the feeder roots, respectively, were ectomycorrhizal with this fungus. *Thelephora* mycorrhizae also occurred on these seedlings.

Another test of the persistence of vermiculite-peat moss inoculum of *P. tinctorius* was done in a pot study in the greenhouse (Marx, unpublished data). Leached inoculum was added to fumigated soil in a 1:8 ratio and incubated without a tree host at soil temperatures of 15, 20, 25, and 30 °C. After two weeks, one, two, and three months at the different temperatures, the infested soil was planted with seed of *P. taeda* and then incubated at 25 °C. Six months later the seedlings were evaluated. The seedlings in soil originally incubated for three months at 30 °C without a tree host had nearly as many ectomycorrhizae formed by *P. tinctorius* as seedlings in soil originally incubated without a host for only two weeks at 15 °C. We concluded from these studies that leached inoculum of *P. tinctorius* can survive in soil under a variety of conditions.

Nursery tests with mycelial inoculum.   Following the test in the microplots (Marx and Bryan 1975), leached vermiculite-peat moss inoculum of *P. tinctorius* was introduced into fumigated soil at state nurseries in Georgia, Florida, and North Carolina (Marx *et al.* 1976). The volume of leached inoculum used in each nursery was 2.8 l/m^2 of soil surface. It was broadcast onto the soil and immediately mixed thoroughly with hand tools into the upper 10 to 12 cm of soil.

In the Georgia nursery, ineffective soil fumigation apparently precluded successful colonization of *P. taeda*, *P. clausa*, or *P. virginiana* seedlings by the introduced inoculum. Problems with soil fumigation were evident by the very high levels of plant parasitic nematodes, phycomycetous root pathogens, and diseased pine seedlings detected at the end of the growing season. Also, the appearance of *T. terrestris* sporophores and mycorrhizae on seedlings as early as the second month after seeding suggested that the residual inoculum of this fungus from the previous year was not seriously affected by the fumigation.

Soil fumigation in the other two nurseries was effective. In the Florida nursery, seedlings of *P. taeda*, *P. clausa*, and *P. elliottii* var. *elliottii* had abundant *Pisolithus* mycorrhizae as early as six weeks after seed germination. Control seedlings of all pines also had a few mycorrhizae formed by naturally occurring fungi at this time. Numerous sporophores of *P. tinctorius* were produced in all plots inoculated with mycelial cultures by August or September. Considerably more sporophores were produced in *P. taeda* and *P. elliottii* plots than in *P. clausa* plots. Seedlings were lifted and evaluated eight months after study installation. Although *Pisolithus* mycorrhizae were formed in abundance, differences in seedling growth and total mycorrhizal development were not detected. Inoculated seedlings of *P. taeda* had 72 per cent mycorrhizal development of which *P. tinc-*

torius formed about one-half. Control seedlings of *P. taeda* had 66 per cent mycorrhizal development, all of which were formed by naturally occurring fungi. On inoculated seedlings of *P. elliottii* var. *elliottii*, *P. tinctorius* formed about eight-tenths of the total mycorrhizal development of 82 per cent. Control seedlings had 73 per cent development by other fungi. *Pisolithus tinctorius* formed over half of the 40 per cent mycorrhizal development on seedlings of *P. clausa*. Control seedlings of *P. clausa* only formed mycorrhizae with naturally occurring fungi on 21 per cent of the feeder roots. There was a positive relationship between the number of sporophores and the amount of mycorrhizae produced by *P. tinctorius* on the different pine species. The fewest number of sporophores were produced in plots of *P. clausa* seedlings which also had the fewest roots colonized by *P. tinctorius*.

In the North Carolina study, mycorrhizal development early in the season on seedlings in inoculated and control plots and the late summer development of sporophores of *P. tinctorius* were similar to that observed in the Florida study. However, in the North Carolina study, stimulation of seedling growth (total fresh weights) by *Pisolithus* mycorrhizae was 140 per cent on seedlings of *P. taeda* and about 100 per cent on seedlings of *P. virginiana* and *P. strobus*. Total mycorrhizal development on all pine species was also increased significantly by mycelial inoculation with *P. tinctorius*. Inoculated seedlings of *P. taeda* had a total of 64 per cent mycorrhizal development, with over nine-tenths formed by *Pisolithus*. Control seedlings had 50 per cent mycorrhizal development by naturally occurring fungi. The inoculated seedlings of *P. virginiana* had a total development of 72 per cent with two-thirds formed by *P. tinctorius*. Control seedlings had 47 per cent of their feeder roots ectomycorrhizal. Seedlings of *P. strobus* had a total development of 47 per cent with about three-quarters formed by *Pisolithus*. Control seedlings only formed 15 per cent mycorrhizae with other fungi. This study showed that following proper soil fumigation, leached vermiculite-peat moss inoculum of *P. tinctorius* can be introduced into soil of conventional tree nurseries and form abundant mycorrhizae on roots of southern pines. The field performance of these seedlings after outplanting on different routine reforestation sites will be discussed later.

Our next nursery research (Marx and Artman 1978) involved comparing the response of *P. taeda* seedlings to inoculation with leached vermiculite-peat moss cultures of *P. tinctorius* and *T. terrestris*. Studies were installed in fumigated soil in two state nurseries in Virginia, one in the coastal plain and the other in the mountains (elevation 580 m). Inoculum of each fungus was applied at a rate of 1.08 l/m^2 of soil surface and mixed thoroughly into the soil. Control

plots received the same amount of vermiculite. This rate of inoculum was used because results from an inoculum density study, to be discussed later, indicated that it is as effective as higher rates. After seeding in April 1975, the seedlings were grown for seven months, lifted, and evaluated.

Only two morphological types of ectomycorrhizae were observed on seedlings in both nursery tests. One type, formed by *T. terrestris*, was observed almost exclusively in *T. terrestris* inoculated plots and the control plots. The other type was formed by *P. tinctorius* and it was observed only in *P. tinctorius* inoculated plots. *Pisolithus* formed nearly nine-tenths of all the mycorrhizae (75 per cent) in both the coastal plain and the mountain nursery. In both nurseries non-inoculated control seedlings had about 46 per cent mycorrhizal development. There were four to five times more sporophores of *T. terrestris* in the plots inoculated with *T. terrestris* in both nurseries than in control plots. Sporophore production by *P. tinctorius* was not recorded, but it was observed in all *Pisolithus* plots. Seedlings from *Pisolithus* and *Thelephora* inoculated plots had 57 and 31 per cent greater fresh weights in the coastal plain nursery and 40 and 20 per cent greater fresh weights in the mountain nursery, respectively, than the controls. Even though *P. tinctorius* and *T. terrestris* formed the same quantity of mycorrhizae on the seedlings, those with *Pisolithus* mycorrhizae were significantly heavier in both nurseries than those inoculated with *T. terrestris*. This indicated that *P. tinctorius*, even though ecologically adapted to soils of low fertility and other adverse conditions, is probably more efficient than *T. terrestris* in maximizing nutrient absorption from soil. It may be that mycorrhizal fungi adapted to poor soils make more efficient use of available nutrients than other fungal symbionts adapted to better soils.

In North Carolina, Krugner (1976) examined the interaction of soil fertility with these two fungi in closer detail on *P. taeda* in a microplot study. Using leached vermiculite-peat moss inoculum of each fungus, fumigated soil was infested with either *P. tinctorius*, *T. terrestris*, an equal mixture of inoculum of both fungi, or autoclaved inoculum of both fungi (control). The inoculum was standardized at 2 l/m^2 of soil surface for all treatments and mechanically mixed into the upper 10 to 12 cm of soil. Fertility treatments of N at 145 kg/ha, NPK at 145, 50, and 100 kg/ha, respectively, and no added fertilizer were imposed on the fungal and control treatments. After eight months the seedlings were lifted and evaluated. Inoculation of soil with either fungus alone or in mixture did not markedly affect seedling growth. Independent of the fungi, both fertilizer treatments significantly increased seedling growth in comparison to

non-fertilized seedlings. Both fertility treatments stimulated the development of *Pisolithus* mycorrhizae whether *P. tinctorius* was added to soil alone or in mixture with *T. terrestris*. *Pisolithus* formed about one-fifth of the total mycorrhizal development of 64 per cent in non-fertilized soil, about one-half of the total development of 80 per cent in the nitrogen-treatment, and about two-thirds of the total mycorrhizal development of 80 per cent in complete NPK treatment. Seedlings in *T. terrestris* and control plots had between 55 per cent and 62 per cent mycorrhizal development in all fertility treatments, including the non-fertilized controls. Krugner concluded that *T. terrestris* did not compete well with *P. tinctorius* for seedling roots under conditions of abundant nutrient availability. He suggested that the inoculum of *T. terrestris* may not have been as vigorous as that of *P. tinctorius*. This latter point is undoubtedly a factor to consider in work of this type. However, *P. tinctorius* may simply be able to compete for roots better than *T. terrestris* in soils with good fertility, at least for the first growing season. Different results may be obtained in soil of higher fertility or in nursery studies of longer duration.

In the previous microplot and tree nursery studies natural recolonization of the fumigated soil by wind-disseminated spores of ectomycorrhizal fungi indigenous to the areas was very rapid and efficient. Usually within a few weeks after seed germination, mycorrhizae were formed on seedlings in previously non-inoculated soil by naturally occurring fungi. Competition existed, therefore, between the natural spore inoculum of the indigenous fungi and the inoculum of the artificially introduced fungi very early in the growing season. In 1974 we were able to examine the significance of soil inoculation with pure mycelial cultures to seedling growth and mycorrhizal development in a new tree nursery having minimal competition from native ectomycorrhizal fungi (Marx *et al.* 1978). The nursery, located in south-eastern Oklahoma, was established on former pasture land in an area surrounded by only a few scattered ectomycorrhizal trees. In 1974 the first crop of *Pinus taeda* seedlings was grown in non-fumigated soil. Recommended rates of fertilizer and pesticides were applied during the growing season. By mid-July the seedlings were stunted and chlorotic; less than 10 per cent of the seedlings had a trace of mycorrhizae. By mid-August thousands of seedlings were dying each week. More fertilizer and pesticides were added but seedling mortality continued. In January 1975, the nearly seven million seedlings were lifted and evaluated. Only 4 per cent had acceptable stem diameters (greater than 3 mm), none met the 15 cm height requirement for planting out, and very few seedlings had mycorrhizae. The nursery managers concluded that the poor growth of seedlings resulted from an insufficient quantity of mycorrhizae.

In April of 1975, a comprehensive study was installed in a new section of this nursery using pure mycelial cultures of *P. tinctorius, T. terrestris,* and *C. graniforme* in both fumigated and non-fumigated soil. Vermiculite-peat moss cultures of *P. tinctorius* and *T. terrestris* were grown for three months and the total volume of each culture vessel was leached and used as inoculum. However, the slower growing *C. graniforme* did not colonize all the mixture in three months; therefore only that part of the substrate with obvious mycelium of *C. graniforme* was leached and used as inoculum. Mycelial inoculum of all fungi was broadcast at a rate of 1.08 l/m^2 of soil surface and mixed into the upper 10 to 12 cm of soil. Control plots received the same rate of vermiculite. *P. taeda* was seeded in April, and during the growing season all seedlings received the same amount of fertilizer and water.

Approximately six weeks after seeding, ectomycorrhizae of *P. tinctorius* and *T. terrestris* were observed on seedlings in their respective plots in both fumigated and non-fumigated soil. Seedlings in other plots had only a few mycorrhizae at this time. By mid-August sporophores of both fungi were detected in their respective plots in fumigated and non-fumigated soil. Seedlings in these plots were vigorous and were nearly twice as large as seedlings in other plots. Mid-season examination of these vigorously growing seedlings showed they had 35 to 40 per cent of their feeder roots mycorrhizal with the respective fungi. Only a few black mycorrhizae of *C. graniforme* were observed on seedlings in the *C. graniforme* plots at this time. By early October, seedlings in the control non-fumigated plots and the fumigated and non-fumigated plots of *C. graniforme* began to grow at normal rates. Concurrent with this new growth was the appearance of *Thelephora* mycorrhizae on the control seedlings and *Thelephora* and *C. graniforme* mycorrhizae on the seedlings in the *C. graniforme* plots. Non-inoculated seedlings in fumigated soil were still stunted and had few mycorrhizae.

In December the 54 000 seedlings in this study were lifted and representative ones evaluated. In fumigated soil, the number of plantable seedlings (greater than 12.5 cm in height and 3 mm in root collar diameter) was increased by 155 per cent over the controls with *T. terrestris,* 140 per cent with *P. tinctorius,* and 77 per cent with *C. graniforme.* The former two fungi also increased the number of plantable seedlings in non-fumigated soil over the non-inoculated controls. Non-fumigated control plots had over twice as many plantable seedlings as fumigated control plots. None of the fungal treatments significantly increased seedling size in non-fumigated soil. However, in fumigated soil *T. terrestris* and *P. tinctorius* increased total fresh weights of plantable seedlings by 125 per cent and *C. grani-*

forme by 24 per cent. Mycorrhizal development was also affected by soil fumigation. In fumigated plots *Pisolithus* mycorrhizae accounted for eight-tenths of the total 60 per cent development. In non-fumigated soil, total development was about 55 per cent with *Pisolithus*-forming over two-thirds of these. Since *Thelephora* occurred naturally in this nursery, it was difficult to make accurate assessments of the value of inoculation with this fungus. Naturally occurring fungi other than *T. terrestris* were less frequent on seedlings on fumigated soil inoculated with *Thelephora* than in non-fumigated soil, but total mycorrhizal development was significantly greater in fumigated (59 per cent) than in non-fumigated (48 per cent) soil. There was just as much naturally occurring *Cenococcum* mycorrhizae on seedlings in the non-fumigated control plots as in the fumigated soil inoculated with *Cenococcum*. *Cenococcum* mycorrhizae accounted for about one-quarter of the mycorrhizae in fumigated inoculated plots and accounted for one-eighth of the mycorrhizae in the non-fumigated inoculated plots. All of the above mycorrhizal assessments of specific fungi were confirmed by surface sterilizing the mycorrhizae and reisolating the fungi on agar medium.

The results of this study revealed several salient points. Mycorrhizal development must occur early in the growing season in order to improve the numbers of plantable seedlings of *P. taeda* and their size. Pure mycelial cultures of specific fungi can be used to correct the erratic occurrence or deficiency of mycorrhizae in both fumigated and non-fumigated nursery soil in a geographic area where few symbiotic fungi occur naturally. Soil fumigation obviously reduces populations of indigenous symbiotic fungi and other micro-organisms, improving the success of artificial soil inoculations with pure mycelial cultures of *P. tinctorius* and *T. terrestris*. Lastly, it appears that *C. graniforme*, owing to its inherently slow growth rate and its adaptation to drought-prone soils, will not effectively colonize roots of pine seedlings in irrigated nursery soils. Perhaps with maintenance of less soil moisture in nurseries where soil colonization by other symbiotic fungi is slow, this fungal symbiont may be effectively maintained on seedling roots.

Rate of mycelial inoculum. During our research with *P. tinctorius* it became apparent that we did not know the least amount of inoculum needed to successfully infest soil and form ectomycorrhizae on seedlings. In many instances the amount of inoculum used in our early tests was dictated by the amount of inoculum available for use at the time. To examine this problem, a study was installed in a tree nursery in Mississippi (Marx, unpublished data). This nursery has been producing good quality pine seedlings for over 25 years that are

usually heavily mycorrhizal with *T. terrestris* and other fungi. *Pisolithus* has also been observed in this nursery. In the spring of 1976, leached vermiculite–peat moss inoculum of *P. tinctorius* was broadcast on fumigated soil at rates of 2.80, 2.16, 1.62, 1.08, and 0.5 l/m^2 of soil surface. Two control treatments were installed; one received 2.8 l/m^2 rate of leached autoclaved inoculum and the other received no inoculum. These were used to delineate any possible physical or chemical effects of the inoculum to the soil and seedling growth. Seeds of *Pinus palustris* and *P. echinata* were planted and the plots mulched. In December, approximately 26 000 seedlings of each pine species were lifted and representative seedlings were evaluated.

Inoculation with *P. tinctorius* at any rate significantly increased total mycorrhizal development from 23 per cent (mean of both control groups) to 34 to 43 per cent on *P. palustris* seedlings. *Pisolithus* mycorrhizae, regardless of the inoculum rate, accounted for one-third of the total development. Significant increases in seedling growth and the number of plantable seedlings were associated with all inoculation treatments. Seedlings of *P. echinata* were also stimulated regardless of inoculum rate. On this species, *Pisolithus* mycorrhizae at the four highest inoculum rates, accounted for about one-third of the total development, but it formed only about one-quarter of all mycorrhizae at the 0.54 l/m^2 rate. *Thelephora terrestris* formed most of the other mycorrhizae. A comparison of seedlings and soil from the two different control treatments showed that the leached inoculum did not affect seedling growth or change chemical (major nutrients) or physical (cation exchange capacity) conditions of the soil. The 1.08 litres per m^2 of soil surface rate was the least amount that could effectively be used for maximum mycorrhizal development in this nursery. This nursery has one of the most rapid colonizations of fumigated soil by *T. terrestris* of any nursery in which we have worked. Therefore, this 1.08 l/m^2 rate may be even more effective in nurseries having a lesser degree of early competition from other fungi. We are currently recommending this rate for purposes of experimentation in properly fumigated nursery soils.

Drying of mycelial inoculum.   The weight and physical nature of pure mycelial inoculum that had been used up to this time caused certain problems. After leaching, the vermiculite–peat moss inoculum had a very high weight to volume index and physically resembled a sticky paste. The inoculum was also very heavy to transport and quite difficult to spread and mix into the soil. A study was conducted to determine the feasibility of drying the inoculum of *P. tinctorius* in order to eliminate these problems (Marx, unpublished data). Vermiculite–peat moss inoculum was leached in tap water, squeezed

to remove excess water, placed 2 cm deep in an aluminium tray and dried to 12 per cent moisture at 28–30 °C for 56 hours in a forced-air oven. In order to ascertain the effects of drying on the efficiency of the inoculum, it was added to soil at different rates. Leached but non-dried inoculum was used in identical fashion for a further comparison. Inoculum was broadcast on fumigated soil in microplots at our nursery in Athens at rates of 2.16, 1.08, 0.54, and 0.27 l/m² of soil surface and mixed 10 cm deep into the soil. Control soil received vermiculite at the highest rate. Seed of *P. taeda* were planted in April 1976 and nearly 8000 seedlings were lifted and evaluated the following December. Dried inoculum was as good as, if not better than, non-dried inoculum for development of *P. tinctorius* mycorrhizae. An average of the three highest rates showed that *Pisolithus* from non-dried inoculum formed less than one-third of the 73 per cent total development while dried inoculum formed nearly half of the mycorrhizae. The lowest rate (0.27 l/m²) of both inoculum formulations formed less than half the amount of *Pisolithus* as the higher rates. Since procedures for soil fumigation are more efficient at our nursery facility than those employed in conventional nurseries, we obtained greater effectiveness at lower rates of both inoculum formulations in this study than obtained from the inoculum rate study in the Mississippi nursery. One reason for the greater effectiveness of the dried inoculum is that it mixes more homogeneously in soil than the paste-like, non-dried inoculum. Removal of excess water from leached inoculum reduced the volume by one-third; drying reduced it further by nearly a third. An initial 3 litre volume of inoculum from culture vessels is reduced to 1.2 to 1.4 litres of usable inoculum after it is leached and dried.

Storage of mycelial inoculum.   Severe limitations would be placed on the broad scale use of this type of inoculum of any fungus if the inoculum could not survive reasonable lengths of storage. There would be few cases where transport of inoculum from the laboratory to the nursery did not entail a period of storage under various temperature conditions. The following study (Marx, unpublished data) investigated the influence of length of storage at different temperatures on the effectiveness of dried and non-dried inoculum of *P. tinctorius*. Inoculum was prepared, as previously described, and 15 ml volumes were stored in test tubes at 5, 23, and 30 °C. At weekly intervals sets of tubes were removed from the incubators. The inoculum was mixed at a 1:8 ratio with fumigated soil and placed in small pots in the mycorrhizal fungus-free growth room. Seed of *P. taeda* were planted and seedlings were evaluated after four months. Non-stored, dried inoculum formed 50 per cent *Pisolithus* mycorrhizae

and the non-dried inoculum formed 57 per cent. This proved initial viability of inoculum. After the first week of storage viability dropped to 48 per cent mycorrhizal development for non-dried and 41 per cent for dried inoculum. This level of viability was maintained for the next seven to nine weeks of storage for inoculum incubated at 5 and 23 °C and for five to seven weeks at 30 °C. Viability decreased significantly after longer periods. The fact that leached and dried inoculum of *P. tinctorius* can be stored for up to nine weeks at refrigeration temperatures and for at least five weeks at warmer temperatures indicates that it is quite durable and should withstand reasonable storage and transportation conditions.

It is apparent from the discussions of research carried out by scientists in various parts of the world that the artificial introduction of specific fungi into nurseries and containers is biologically feasible. Published reports indicate that inoculation programmes developed in Austria and Argentina are on a quasi-operational level for practical application. In the United States, a test programme is currently underway to determine the feasibility of producing inoculum of *P. tinctorius* for commercial uses. In the spring of 1977, Abbott Laboratories, Long Grove, Illinois, produced a dried, vermiculite-peat moss inoculum of *P. tinctorius* which we tested in 19 identical nursery experiments in 15 states of the South-East, South, and South-West. Seven different species of *Pinus* and *Quercus rubra* were involved. In each experiment different rates (1.62, 1.08, and 0.54 l/m^2 of soil surface) of the Abbott-produced inoculum and one rate (1.08 l/m^2) of dried inoculum produced in our laboratory were compared. This inoculum was further evaluated in seedling container programmes in five different states involving eight species of *Pinus*, *Pseudotsuga menziesii*, and *Tsuga heterophylla*. From September 1977 to March 1978 over 150 000 seedlings were lifted and representative seedlings were evaluated in Athens. Although the results were erratic and somewhat inconsistent, they showed that the dried, vermiculite-peat moss inoculum of *P. tinctorius* can be produced in large volumes in industrial fermentors and is functional in forming mycorrizae on seedlings. In the spring of 1978 our tests were expanded to include the entire United States using an improved inoculum production method. 33 bare-root and 11 container nursery tests are currently underway. These tests involve all major ectomycorrhizal tree species grown in the United States. Studies will be terminated in the bare-root nurseries after one, two, or three years depending on the tree species under test. We are very optimistic about the biological value of this commercially produced inoculum. If this product form of *P. tinctorius* inoculum proves to be functional, Abbott Laboratories has the fermentor capacity to potentially

produce hundreds of thousands of litres for application in world forestry.

Fungus selection criteria and maintenance of pure cultures

The most important first step in any nursery inoculation programme is the selection of the fungi (Bowen 1965; Marx 1977a; Mikola 1973; Moser 1963; Trappe 1977). The physiological differences that exist between mycorrhizal fungi can be used as criteria for their selection. The importance of each of the following criteria will vary according to the needs of the different inoculation programmes in different locations. Therefore, criteria will not be ranked in this discussion.

Host specificity

One criterion is host specificity. The consistent association of certain fungi for only a few specific tree hosts is well documented in the literature. Many other fungi are associated with a great number of different tree hosts (Marx 1977b; Stevens 1974; Trappe 1962). It is imperative, therefore, that the candidate fungi exhibit the physiological capacity to form mycorrhizae on the desired hosts. There is another aspect to this criteria, however. It is not sufficient to simply select a fungal species and then obtain an isolate for testing. Several isolates from different tree hosts and geographic regions should be used. This point has been stressed by Moser (1958c) and demonstrated by Theodorou and Bowen (1970) with isolates of *Rhizopogon luteolus*. We have obtained isolates of *P. tinctorius* from different species of oaks and compared them with isolates from pine in the mycorrhizal fungus-free room and in the microplot nursery on *Quercus rubra* seedlings (Marx, unpublished data). The pine isolates formed abundant mycorrhizae in the growth room and nursery. Some oak isolates formed a few mycorrhizae; some isolates did not form mycorrhizae at all. All isolates were similar in age and had comparable pigmentation and rates of vegetative growth in agar medium.

Growth in pure culture

Another criterion is the ability of the selected fungi to grow in pure culture; many ectomycorrhizal fungi will not. A variety of culture media (Moser 1958b; Stevens 1974; Trappe 1962) and methods of isolation (Palmer 1971) can be used to obtain pure cultures of the selected fungi. Ideally, the fungi should be able to grow rapidly (Moser 1959). Once cultures of the selected fungus have been obtained they must be maintained in a viable condition. Takacs (1967) recommends subculturing the stock cultures of the fungi every 60 days in order to retain vigour. Moser (1958b) stressed the need to

subculture every two to five weeks, depending on fungus species, especially those to be used in current inoculation programmes. If the cultures are not subcultured frequently they exhibit poor growth and loss of pigment. He recommends growing declining cultures on a different medium to rejuvenate them. We found that continuous culturing of certain fungi on agar media for several years frequently decreased mycelial growth rate and the capacity to form mycorrhizae on pine. Changes in adaptive enzyme systems during continuous vegetative growth on synthetic medium probably accounts for this loss of ability to symbiotically infect the host roots. Mycelial agar discs cut from plate cultures and stored in sterile distilled water at 5 °C can be held for up to three years without loss of these physiological traits (Marx and Daniel 1976). The technique does not work for all fungi but is worthy of testing. The storage of cultures in a dormant physiological state should reduce, if not eliminate, shifts in adaptive enzyme systems. A certain amount of caution, therefore, must be used in evaluating fungi maintained in continuously growing, pure cultures for extended periods of time. We had isolates of *P. tinctorius* grown in continuous culture for 15 years that were still highly pigmented and grew at rates comparable to that achieved shortly after their isolation from sporophores. In 1974 these isolates lost their capacity to form mycorrhizae on pine. One of these (isolate 29) had been used successfully by us in several earlier studies (Marx, Bryan, and Davey 1970) since its original isolation. We have found, however, that our best isolates of *P. tinctorius* are those that are cycled back through their host every year or two and then reisolated from sporophores or directly from mycorrhizae. Our main isolate of *P. tinctorius* currently under test with Abbott Laboratories was first isolated in 1967 from a sporophore under a mature *P. taeda* growing in Georgia. This isolate has been rejuvenated by cycling it through pine hosts every one or two years. Today, it grows faster and forms more mycorrhizae on pine and oak than it did in 1967. It also has formed mycorrhizae on a variety of host species that other recently cultured isolates have not. A parent culture of this isolate maintained in continuous culture will form few mycorrhizae at this time.

If spores of a selected fungus are to be used for inoculum, then the ability of this fungus to grow well in pure culture is of little importance. However, growth in pure culture may be useful if it is to be reisolated from mycorrhiza to confirm its identity.

Once the growth potential of a fungus has been confirmed it is important to confirm its capacity to withstand physical manipulation (leaching, drying, soil incorporation, colonization by saprophytes, etc.). Producing large quantities of inoculum of a fungus is of little value if the fungus cannot survive the rigours of various manipulations

essential to inoculation of soil. Certain fungi grow readily in vermiculite-peat moss medium but cannot survive the leaching procedure or soil inoculation. If we had not studied the colonization of non-leached mycelial inoculum of *P. tinctorius* by various saprophytic micro-organisms and rectified the problem by leaching, we could have easily concluded that *P. tinctorius* was not a good fungal candidate for any inoculation programme.

Fungus adaptability

Another criterion is the adaptation of the selected fungus to the major type of site on which the seedlings are to be outplanted. Of equal importance is the ability of the fungus to survive and grow under cultural conditions used in nurseries. According to Trappe (1977), the ecological adaptability of an ectomycorrhizal fungus hinges on the metabolic pathways it has evolved to contend with environmental variation. Extremes of soil and climatic factors, antagonism from other soil organisms, pesticide application, physical disruption of mycelium from nursery operation, and the abrupt adjustment from a fertilized and irrigated nursery soil to an uncultivated planting site with all of its stresses are only a few of the environmental variations to which the selected fungi must adapt.

The effect of temperature on different species and ecotypes of ectomycorrhizal fungi is perhaps the most widely researched environmental factor. Upper and lower temperature limits of the candidate fungi should be determined. Moser (1958d) studied the ability of fungi to survive long periods (up to four months) of freezing ($-12\,^{\circ}$C) and to grow at low temperatures (0-$5\,^{\circ}$C). He found that high elevation ecotypes of *Suillus variegatus* were not damaged after freezing for two months, but valley ecotypes were killed after freezing for only five days. Although not as striking, similar results were reported with *S. tridentinus*, *S. plorans*, and *Gomphidius rutilus*. In low temperature growth studies, none of the species of *Amanita* grew at 5 or $0\,^{\circ}$C. An interesting observation was that certain species and ecotypes which survived freezing for extended periods did not grow at low temperatures. Generally, he found that mountain ecotypes and species had much lower temperature optima than lowland ones. Even after several years in pure culture at 20 to 23 $^{\circ}$C, the low temperature fungi still maintained optima near 15 $^{\circ}$C. *Pisolithus tinctorius* not only survives and grows well at unusually high temperatures, but also it grows at 7 $^{\circ}$C and survives in frozen soil (Marx, Bryan, and Davey 1970, Marx and Bryan 1971, 1975). High temperature tolerance makes *P. tinctorius* an excellent candidate for testing in the tropics (Momoh and Gbadegesin 1975). *Rhizopogon luteolus* apparently is not suitable for inoculation programmes because of its inability to

survive or grow at the high soil temperatures common to this area (Momoh 1973).

Reaction of the candidate fungi to soil moisture, organic matter, and pH are also important traits to consider. *Cenococcum graniforme* is not only drought tolerant but forms mycorrhizae in natural soils ranging in pH from 3.4 to 7.5 (Trappe 1964). We have observed *Pisolithus* mycorrhizae on pine in drought-prone coal spoils ranging in pH from 2.6 to as high as 8.4. Trappe (1977) has observed several species of fungi which form ectomycorrhizae in well-rotted conifer logs with a pH of 4.0 or lower in the Pacific North-west of the United States. Levisohn (1965) observed in England that *Suillus bovinus*, an excellent mycorrhizal fungus on spruce, naturally occurs in nursery soils containing abundant organic matter. Unfortunately the fungus disappears from the roots of spruce planted on sites having low organic matter. Its potential value in inoculation programmes would appear to be restricted to sites with high levels of organic matter.

Value of hyphal strands

Another criterion by which candidate fungi should be evaluated is their capacity to form hyphal strands in pure cultures and in soil. Bowen (1973) showed that nutrient uptake, especially phosphorus, is greater in fungi that produce hyphal strands. In Australia, one of the initial criteria for selection of fungi is their ability to produce hyphal strands under a wide range of conditions. Although research data is lacking we believe that the abundant hyphal strands produced by *P. tinctorius* not only enhance nutrient absorption, but increase its survival potential under adverse conditions. Yellow-gold hyphal strands of *P. tinctorius*, easily visible to the naked eye, have been traced through highly toxic and hot coal spoils as far as 4 m from seedlings to sporophores by Schramm (1966) and others (Marx 1977a). On an exposed borrow pit in South Carolina we traced hyphal strands of *P. tinctorius* over 3 m from mycorrhizal roots of *P. palustris* to sporophores.

Aggressiveness of fungus

Another extremely important criterion is the aggressiveness of the candidate fungus to feeder roots. The fungus should have the capacity to form abundant mycorrhizae as soon as feeder roots are formed. It must be able to maintain superiority over naturally occurring fungi in the nursery. Aggressiveness is best evaluated by making quantitative assessments on the amount of mycorrhizae formed by the introduced fungus at different intervals of time. Quantitative assessments are the only valid parameter which can be used to judge the effectiveness of inoculations. We have found that maximum

benefit of *Pisolithus* mycorrhizae is achieved on pine seedlings when at least two-thirds of all the mycorrhizae on the seedlings are formed by *P. tinctorius.*

Field performance of seedlings with specific ectomycorrhizae

The ultimate proof of the value of inoculation of bare-root or container grown nursery seedlings with specific fungi is their performance under diverse field conditions. Meaningful conclusions can only be obtained from properly designed, installed, and maintained field experiments which include periodic tree measurements and mycorrhizal assessments conducted over several years. Only limited field data of this type is available in the literature. Moser (1963) reported that spruce seedlings with mycorrhizae formed by *Phlegmacium glaucopus* survived and grew better than comparable non-mycorrhizal seedlings on a 2100 m altitude forest site in Austria. In another test, four-year-old nursery grown seedlings of *Pinus cembra* with few mycorrhizae were planted on a 2100 m altitude site. These seedlings were inoculated (apparently at planting time) with an equal mixture of pure mycelial inoculum of *Suillus plorans, S. placidus, Paxillus involutus,* and *Amanita muscaria,* a mixture of mycelial inoculum of these four mycorrhizal fungi contaminated with *Penicillium, Mucor,* and bacteria, or no inoculum. After three years the mixed inoculum (either pure or half-pure) of the symbiotic fungi stimulated height growth and increased the number of healthy seedlings by 65 per cent over the non-inoculated controls. Half-pure inoculum of the fungi was only slightly less effective in stimulating seedling survival and growth than the pure mycelial mixture. An assessment of mycorrhizal development was not reported in this study.

Puerto Rico

In Puerto Rico in 1965, Vozzo and Hacskaylo (1971) outplanted seedlings of *Pinus caribaea* from one of the container nursery experiments on a sandy loam site. Unfortunately, damage to seedlings by vandals and cattle shortly after planting resulted in study termination after only six months. Results showed, however, that mycorrhizae formed by *Suillus cothurnatus, Rhizopogon roseolus, Corticium bicolor,* and by unidentified fungi in natural soil inoculum stimulated height growth of the seedlings over both fertilized and non-fertilized, non-mycorrhizal seedlings. Regardless of fertility, non-mycorrhizal seedlings were chlorotic and stunted. At the time of planting, 75 per cent of the seedlings inoculated with pure mycelial cultures and 95 per cent of the seedlings inoculated with the natural inoculum had ectomycorrhizae. Apparently the degree of development on individual

seedlings was not determined. Their results indicated that *S. cothurnatus*, *R. roseolus*, and *C. bicolor* in pure mycelial inoculum can be used in Puerto Rico for the establishment of *P. caribaea*.

Australia

In Australia, Theodorou and Bowen (1970) installed two field experiments with *Pinus radiata* seedlings. In the first test, one-week-old seedlings were transplanted into fumigated potting mixture contained in small wooden veneer tubes. Each tube contained a 10 g layer (3 cm deep in tubes) of pure mycelial inoculum of either *Suillus granulatus*, *S. luteus*, or two isolates of *Rhizopogon luteolus*. Sterile medium was added to control tubes. The seedlings were grown for four months in a greenhouse and then hardened off for an additional three months in the open prior to outplanting. The seedlings were planted in 1966 on a loamy soil field site some 200 m from an established stand of *P. radiata*. At planting, all inoculated seedlings were 7 cm tall and had about 25 per cent mycorrhizal development. Control seedlings were 6 cm tall with only a trace of mycorrhizae. The field design was a randomized design with three blocks. A buffer row surrounded each plot within each block. Significant differences in height occurred as early as six months after planting. Seedlings with *S. granulatus* or *R. luteolus* mycorrhizae were about 46 per cent taller (13.9 cm) than control seedlings (9.5 cm). Seedlings with *S. granulatus* were also noticeably greener than seedlings of other treatments. All seedlings had a healthy green colour after 28 months. Following a summer drought, nearly three times more control seedlings had died (13 per cent) than did those inoculated with *S. granulatus* or *R. luteolus* (3-5 per cent). 20 per cent of the seedlings with *S. luteus* died during the drought. All inoculated seedlings were significantly taller than controls after eight months. After 32 months, the rate of height growth of seedlings with *S. granulatus* mycorrhizae was significantly greater than control seedlings. At 36 months the rate of growth was similar, but differences in height that developed earlier were still evident. Root evaluations revealed that differences in growth due to the different fungi were related to the degree of ectomycorrhizal development. *Suillus granulatus* formed significantly more mycorrhizae (81 per cent) during the 36 months than did the two *R. luteolus* isolates (78 per cent), *S. luteus* (68 per cent), or the control seedlings (65 per cent). White mycorrhizae typical of those produced by the test fungi dominated inoculated seedling roots and a brown-type was observed on the control seedlings.

In their second field test Theodorou and Bowen (1970) grew *Pinus radiata* seedlings as before, except *S. granulatus*, *S. luteus*, *R. luteolus*, and an unidentified isolate obtained from mycorrhizae of nursery

seedlings were used. At planting all seedlings were 6 to 7 cm tall. Inoculated seedlings had 16 to 23 per cent mycorrhizal development and control seedlings had none. These seedlings were outplanted 900 m from an established *P. radiata* stand. After 23 months, there were no significant differences in seedling heights or development of mycorrhizae between treatments. The control seedlings had a similar amount of mycorrhizae (76 per cent) to the inoculated seedlings (69 to 82 per cent). A white ectomycorrhizal type which apparently occurred naturally on this site was observed early on inoculated and control seedlings. This natural colonization of roots of control seedlings obviously minimized the effect of inoculations. The authors stressed the need for larger field plots, more extensive buffers between plots, and test sites which do not contain ectomycorrhizal fungi of *P. radiata* for future studies. They feel these conditions are essential to valid testing of the significance of the various fungi. In spite of problems in the second study, their results suggested that pure mycelial cultures of *S. granulatus* and *R. luteolus* can be used to form abundant mycorrhizae and generally improve field performance of *P. radiata* seedlings. In the first test, *S. luteus* improved field performance but to a lesser degree.

Adverse sites in United States

Beginning in 1973, field tests were conducted in the United States to ascertain the value of mycorrhizae formed by *Pisolithus tinctorius* and other fungi for improving survival and growth of pines on adverse sites. Since most of this data was summarized recently (Marx 1977a), only a few examples will be briefly discussed and updated here. One of our first tests was installed in Kentucky on a very toxic (pH 3.8) coal spoil that had been unsuccessfully planted with pine seedlings several times. Seedlings of *Pinus virginiana* were produced in our nursery with *Pisolithus* and *Thelephora* mycorrhizae using methods described earlier (Marx and Bryan 1975). The seedlings were graded to similar heights and root collar diameters; all had about 75 per cent mycorrhizal development. Seedlings inoculated with *P. tinctorius* had two-thirds of this amount formed by *P. tinctorius*. All other mycorrhizae were formed by *T. terrestris*. The field design was random with five blocks. Test plots within each block were separated by a 4 m non-planted border. After two years only two of the 160 seedlings with *Thelephora* mycorrhiza survived, whereas 78 of the 160 seedlings with *Pisolithus* mycorrhiza survived. More significant was the growth of *Pisolithus* seedlings which produced an average seedling volume* of 130 cm^3 compared to the two *Thelephora* seedlings with an average of 3 cm^3. This volume of

*Seedling volume (cm^3) = (root collar diameter, cm)^2 × height, cm.

Thelephora seedlings was the same as that measured at planting, indicating that these seedlings did not grow during this two-year period.

A similar planting with five blocks was installed on a coal spoil (pH 3.4) in Virginia with *P. taeda* seedlings produced as before. Unfortunately, trees destroyed by vandals precluded accurate assessments of survival. Growth measurements after two years showed that seedlings with *Pisolithus* mycorrhiza has an average seedling volume of 962 cm^3 and those with *Thelephora* mycorrhiza had a volume of 379 cm^3. The last example of a coal spoil study was installed on another toxic (pH 3.9) site in Kentucky. This spoil was unsuccessfully planted twice with nursery seedlings of *P. taeda*. Seedlings of *P. taeda* and *P. echinata* were produced as before with *Pisolithus* and *Thelephora* mycorrhizae and graded to similar sizes and ectomycorrhizal development. The field design was randomized with five blocks. After two years survival was not influenced by treatments, but growth was strongly affected. *Pinus taeda* seedlings with *Pisolithus* mycorrhiza had plot volume indices* of 13 000 cm^3 and those with *Thelephora* had 2000 cm^3. *Pinus echinata* seedlings with *Pisolithus* mycorrhiza had plot volumes of 3600 cm^3 compared to *Thelephora* seedlings with a volume of 700 cm^3 (Marx and Artman 1979).

We prefer this plot volume index (PVI) parameter because it integrates survival, height, and root collar diameters into a single value for comparison. It also represents an accurate measure of the response of all seedlings to treatment. We have found that height measurements alone give poor representations of pine seedling performance.

In all the field tests on coal spoils yearly root evaluations were made. The results confirmed that *P. tinctorius* is ecologically adapted to these harsh sites. Without exception, seedlings with *Pisolithus* mycorrhiza at planting had new roots totally colonized by this fungus. Colonization was so prolific that after the second year hyphal strands were easily detected in the spoil material with the unaided eye as far as 3 m from the young seedlings. Production of sporophores of *P. tinctorius* in the test plots was also prolific. In one *Pisolithus* plot (49 m^2) of *P. taeda*, 83 sporophores were collected during one visit. In contrast, on seedlings that initially had *Thelephora* mycorrhizae new root growth was minimal and only a few were colonized by *Thelephora* at the end of the first year. Many of the original *Thelephora* mycorrhizae (mycorrhizae located on the original root system) were necrotic and only a few visually appeared to be functional. After the second year, a low incidence (three to five per cent) of *Pisolithus* mycorrhizae was detected on newly formed roots on one site; *Thelephora* had spread slowly to new roots on the two other

*Plot volume index, cm^3 (PVI) = (root collar diameter, cm)^2 × height, cm × No. surviving tree seedlings per plot.

sites. Sporophores of *T. terrestris* were only occasionally observed in *Thelephora* plots.

Fourth year data were recently collected and evaluated from the tests on coal spoils in Virginia and the last site in Kentucky. Differences in growth were greater after four years than those measured after two years. One interesting observation was made after the severe winter of 1976-7. Winter scorch of needles was severe on all seedlings on both sites. Seedlings with *Pisolithus* mycorrhiza, however, recovered from this severe needle browning at least six weeks earlier in the spring than seedlings with *Thelephora* mycorrhiza. This earlier recovery undoubtedly gave seedlings with *Pisolithus* mycorrhiza a longer active growth period than seedlings with *Thelephora* mycorrhiza.

A variety of field tests have also been installed on other types of adverse sites (Marx 1977a). Only a few of these will be discussed here. Kaolin spoils are created by strip mining. They are nutrient deficient, reflect sunlight from their light colour, and usually are drought-prone and highly compacted, but most lack the toxic characteristics of coal spoils. In 1975, *P. taeda* seedlings with mycorrhizae formed by either *P. tinctorius*, *T. terrestris*, or *C. graniforme* were produced in our mycorrhizal fungus-free growth room by Otrosina (1977). Seedlings inoculated with *P. tinctorius* had 85 per cent *Pisolithus* mycorrhizal development. Those inoculated with *Cenococcum* had 20 per cent *Cenococcum* and 30 per cent *Thelephora* (contaminant) mycorrhizae. Seedlings inoculated with *T. terrestris* had 85 per cent *Thelephora* mycorrhizae. These seedlings were outplanted in central Georgia on two different kaolin spoils. Both sites were covered with 7.5 cm of forest soil prior to planting. This is a recommended practice to assist in reclamation of the spoils. Treatments were arranged in a random block design with four blocks. Half of the seedlings were fertilized with 170 g of 10:10:10 NPK fertilizer per seedling at planting and the other half received no fertilizer. Only first year data are available. On one site, seedlings with *Cenococcum* mycorrhizae survived better in the fertilized (83 per cent) than in the non-fertilized (55 per cent) plots, but seedling growth was similar. Survival (65 and 73 per cent) and growth of seedlings with *Thelephora* mycorrhizae were not affected by fertilization. Generally, seedlings with *Cenococcum* grew better than seedlings with *Thelephora* mycorrhiza. In the non-fertilized plots, seedlings with *Pisolithus* mycorrhiza were significantly larger and survived better (73 per cent). Overall seedling growth on the second site was generally greater than growth on the first site. Fertilization significantly improved height growth but specific mycorrhiza had no effect (only *Pisolithus* and *Cenococcum* tested). Root evaluation at the end

of the growing season revealed that all introduced fungi persisted on roots of their respective seedlings. However, on the first site the degree of mycorrhiza developed by each fungus decreased; on the second site, the degree of development was greater for both *Pisolithus* and *Cenococcum* than on the other site. This author also concluded that ectomycorrhizal fungi, such as *P. tinctorius* and *C. graniforme*, ecologically adapted to adverse soil conditions, afford improved survival and growth to pine seedlings on kaolin spoils over seedlings with mycorrhizae formed by fungi such as *T. terrestris*. In earlier work on kaolin spoils (Marx 1977a), hyphal strands of *Pisolithus* were found to spread very rapidly through the spoil material. In one study, spread was so rapid that by the end of the first growing season hyphal strands had grown nearly 2 m from inoculated seedlings and had formed mycorrhizae on control seedlings planted in adjacent rows. The integrity, therefore, of the ectomycorrhizal treatments was lost. This rapid spread between rows brought about changes in subsequent experimental designs. Since 1974, we plant seedlings with different mycorrhizal fungi in discrete plots separated by at least a 2 m non-planted border. Frequently, if space is available, a 4 m strip is left non-planted.

Severely eroded sections of the Copper Basin of Tennessee were also used as outplanting sites. Since the 1840s thousands of hectares of productive forest were decimated by cutting timber and using the wood in heap roasting of mineral ores. This roasting produced SO_2 which further damaged vegetation. Severe sheet erosion followed this destruction of vegetation. Air quality was improved in 1964 and reforestation efforts began, but with little success. The surface soil in most places in the basin is gone, leaving nothing more than exposed parent material. The soil has low levels of available nutrients, high temperatures during summer months, and poor internal water drainage, but does not contain levels of any element toxic to pines. Root examinations of pines planted in the basin reveal two obvious forms of ectomycorrhizae. *Thelephora* mycorrhizae and its sporophores occur sporadically. It was probably introduced on roots of the outplanted seedlings from a nursery. The other type, formed by *Pisolithus*, occurs naturally on pines and oaks on the perimeter of the Basin and was probably introduced by wind-borne basidiospores.

Tests were installed at two locations in the Basin in March 1974 (Berry and Marx 1978). Seedlings of *Pinus taeda* and *P. virginiana* were produced in our microplot nursery with *Pisolithus* and *Thelephora* mycorrhizae as described earlier. All seedlings were graded to similar sizes and degrees of mycorrhizal development. Those inoculated with *Pisolithus* had over two-thirds of the total of 70 per cent mycorrhizal development formed by *Pisolithus* and seedlings inocu-

lated with *Thelephora* had about 70 per cent development by *Thelephora*. Prior to planting, the test sites were levelled and the soil was ruptured (subsoiled) to a depth of 60 cm to destroy hardpans and to allow roots and water to more readily penetrate the soil. Fertilizer (672 kg/ha of 10:10:10 NPK) and dolomitic limestone (4480 kg/ha) were broadcast and disced into the soil of all plots. The trees were planted in a randomized design with three blocks. Each plot was surrounded by a border row of trees and a 2 m non-planted strip. After two years, survival of both pine species (88 to 99 per cent) was not affected by mycorrhizal treatments. *Pisolithus* mycorrhizae significantly increased height and stem diameters of both pine species on one site. Seedling volumes of *P. taeda* were 93 per cent and *P. virginiana* were 90 per cent greater than those of comparable seedlings with *Thelephora* mycorrhiza. Unfortunately excessive experimental variations precluded statistical significance on the second site, even though *Pisolithus* mycorrhiza increased seedling volume by 45 per cent for *P. taeda* and 26 per cent for *P. virginiana* seedlings. Both fungi persisted well on roots, since root evaluations showed that they were still dominant on their respective seedlings. Sporophores of *P. tinctorius* were also produced in great abundance in the *Pisolithus* plots.

The last example of research done on adverse sites is from a borrow pit in the lower piedmont of South Carolina (Ruehle, unpublished data). A borrow pit is an area from which soil was removed (borrowed) for use in construction. This eight hectare site was created in the 1950s by vertical removal of from 1 to 5 m of soil. The resulting surface material had physical and chemical characteristics very similar to those of kaolin spoils. In 1955, the site was planted with nursery seedlings of *P. taeda*. By 1975, most of the trees were less than 2 m tall and very chlorotic; root penetration was very restricted. *Pisolithus* occurred naturally on many of these trees. Prior to study installation in 1975, the trees were removed and the site was levelled. The area was then subsoiled as described earlier. Fertilizers (560 kg/ha of 10:10:10 NPK) and dolomitic limestone (2240 kg/ha) were broadcast on half the plots and the other half received a 1 cm layer of dried sewage sludge. All plots were disced and seeded to grass. In 1976, seedlings of *P. taeda* were grown in styrofoam containers with vermiculite–peat moss substrate. Prior to seeding, the substrate in one-third of the containers was mixed 8:1 with leached, non-dried pure mycelial inoculum of *P. tinctorius*. These seedlings were grown in the greenhouse. One-third were grown in the greenhouse without artificial inoculation for natural colonization by *T. terrestris*. The remaining one-third were grown in the mycorrhizal fungus-free growth room in a non-mycorrhizal condition. All seedlings were

watered as needed and fertilized lightly. After four months, all seed-
lings, regardless of treatment, were about 10 cm tall. Those inocu-
lated with *Pisolithus* had about 20 per cent mycorrhizae formed by
Pisolithus and 30 per cent mycorrhizae formed by naturally occur-
ring *T. terrestris*. The second group had a total of 65 per cent mycor-
rhizal development, all formed by *T. terrestris*. The third group from
the growth room lacked mycorrhizae. Seedlings were planted in
November 1976 in a randomized design with five blocks. Each plot
was surrounded by a border row of seedlings and a 4 m non-planted
space.

Only data from one growing season are available. In the sludge
amended plots, survival was 90 per cent for pine seedlings with a
mixture of *Pisolithus* and *Thelephora* mycorrhiza, 75 per cent for
those with just *Thelephora* mycorrhiza, and only 62 per cent for
non-mycorrhizal seedlings. The PVIs were 1702 cm^3 for seedlings with
Pisolithus mycorrhiza, 361 cm^3 for those with *Thelephora*, and only
104 cm^3 for those without mycorrhiza. The differences due to *Piso-
lithus* mycorrhiza in comparison to *Thelephora* and non-mycorrhizal
seedlings represent increases of 372 and 153 per cent, respectively. In
the fertilized plots similar results were obtained but they were not as
striking. Survival was 98, 89, and 88 per cent, respectively, for seed-
lings with *Pisolithus*, *Thelephora*, or no mycorrhizae. The correspond-
ing PVIs were 75, 61, and 27 cm^3. Root evaluation data are not
available at this time.

These results not only show that seedlings with mycorrhizae sur-
vive and grow better than seedlings lacking mycorrhizae, but they
also show that seedlings with *Pisolithus* mycorrhiza are better adapted
to adverse soils in borrow pits even after amendments with sludge or
fertilizers.

We can conclude from these field studies on adverse sites that
reclamation and reforestation of such sites can be expedited by using
pine seedlings tailored with mycorrhizae formed by fungi capable of
growing under adverse conditions. Thus, the planting of seedlings
with root systems physiologically and ecologically adapted to accom-
modate the adversities of the planting site can be an important biolo-
gical tool in reforestation. In reality, however, sites such as coal and
kaolin spoils and borrow pits are not the only adverse sites created
by the activities of man. Many reforestation sites, especially those
which have been intensively site prepared (stump shearing, root
raking, slash removal, burning, discing, etc.), are temporarily adverse
(Schultz 1977). Until vegetation is re-established by either natural
or artificial means, the mineral soils are often exposed and subject
to broad fluctuations of temperature, moisture, and fertility, as well
as to erosion and compaction (Haines, Maki, and Sanderford 1975).

These are adverse soil conditions, however temporary, to which root systems of newly planted seedlings will be exposed. If these soil conditions are extreme, the survival and early growth of seedlings with mycorrhizae formed by nursery adapted fungi, i.e. *Thelephora*, may be unduly affected. This point could explain certain reforestation failures in the past on sites which have been intensively prepared.

Routine sites in United States

After considering these points, we installed several studies on routine reforestation sites to compare the effects of *Pisolithus* and *Thelephora* mycorrhiza on establishment and early growth of various pines. Since 1974, nearly 75 000 seedlings have been experimentally outplanted on a variety of reforestation sites. In most studies, seedlings were graded so that different amounts of *Pisolithus* and *Thelephora* mycorrhizae were treatment variables. The following are results of one such study (Marx, Bryan, and Cordell 1977). Pine seedlings were produced in Florida and North Carolina nurseries with *Pisolithus* and naturally occurring *Thelephora* mycorrhizae. The nursery phase of this study was described earlier (Marx *et al.* 1976). The use of basidiospores and pure mycelial inoculum of *P. tinctorius* resulted in seedlings with different quantities of *Pisolithus* mycorrhizae. In the Florida nursery, seedlings of *P. taeda*, *P. elliottii* var. *elliottii*, and *P. clausa* were graded to equal size and to 65 per cent mycorrhizal development. Those inoculated with mycelial inoculum had three-quarters of this 65 per cent formed by *Pisolithus* and those inoculated with basidiospores had about one-third of this amount formed by *Pisolithus*. Remaining mycorrhizae on these seedlings and the control seedlings were formed mainly by *Thelephora terrestris*. Three sites in Florida, recently clearcut of pines, were site prepared and all slash was burned exposing mineral soil. One site was a deep sand ridge; one was a palmetto flatwood. The third site was also a flatwood site but it was planted to only *P. elliottii* var. *elliottii* with abundant *Pisolithus* or *Thelephora* mycorrhizae. No intermediate amount of *Pisolithus* was used. This study also involved a fertility variable of 90 kg/ha of both nitrogen and phosphorus.

In the North Carolina nursery, seedlings of *P. taeda*, *P. virginiana*, and *P. strobus* were graded to equal size and also to 75 per cent mycorrhizal development. Generally, seedlings inoculated with mycelial inoculum had at least eight-tenths of the 75 per cent total development formed by *Pisolithus* and those inoculated with basidiospores had between one-tenth to two-thirds of the total mycorrhizae formed by *Pisolithus*. The two test sites in North Carolina were cleared of pine and oak, site prepared, and all slash was piled and burned. One site was considered a good reforestation site because

of the presence of 25 cm of top soil. The other was considered poor because it was an eroded slope.

Seedlings on four sites were planted in a random design with five blocks. The fertilizer–mycorrhiza study had only three blocks. Plots within blocks were separated by at least a 3 m non-planted strip.

After two years, seedlings with the greatest quantity of *Pisolithus* mycorrhizae at planting generally survived and grew better than seedlings with the same amount of mycorrhizae formed by *T. terrestris*. Seedlings with less *Pisolithus* mycorrhizae were usually intermediate between these two seedling groups in survival and growth. In North Carolina, abundant *Pisolithus* mycorrhizae significantly increased PVI of both *P. virginiana* and *P. taeda* by about 25 per cent on the good site and by about 50 per cent on the poor site in comparison to seedlings with only *Thelephora* mycorrhizae. *Pinus taeda* seedlings with less *Pisolithus* and more *Thelephora* mycorrhizae (from basidiospores) on the better site had a 30 per cent greater PVI than did the *Thelephora* seedlings; they were not different on the poor site. Greater differences occurred on seedlings of *P. strobus*. Those with abundant *Pisolithus* mycorrhizae had five times greater PVI than seedlings with *Thelephora*. This test with *P. strobus* is somewhat unusual since these seedlings were outplanted after only one growing season in the nursery instead of the normal two growing seasons.

In Florida, the results were similar. On both sites *P. taeda* and *P. elliottii* var. *elliottii* seedlings with abundant *Pisolithus* mycorrhizae had significantly greater PVI (16 to 51 per cent) than *Thelephora* seedlings. On the palmetto flatwood, *P. taeda* seedlings with only one-third of the complement of mycorrhizae formed by *Pisolithus* also had a PVI that was 46 per cent greater than *Thelephora* seedlings. The intermediate amount of *Pisolithus* on seedlings of *P. elliottii* var. *elliottii* did not have an effect on growth. The most significant results were obtained with *P. clausa*. Seedlings with abundant *Pisolithus* had PVIs on both sites that were from 270 to 445 per cent greater than seedlings with *Thelephora*. The greatest difference in survival and growth occurred on the flatwood site which is considered off-site for *P. clausa*. In the fertility study, *P. elliottii* var. *elliottii* seedlings with *Pisolithus* mycorrhiza in the fertilized plots were the same size as seedlings with *Thelephora*. However in the non-fertilized plots, seedlings with *Pisolithus* mycorrhiza had a PVI that was 175 per cent greater than comparable seedlings with *Thelephora* mycorrhiza. Yearly root evaluation for two years of representative seedlings on the various sites revealed that *Pisolithus* persisted well, especially on those sites where it stimulated the most seedling growth. On all sites, particularly the better ones, other ectomycorrhizal fungi such as *Cenococcum graniforme* or an unidentified *Rhizopogon* were
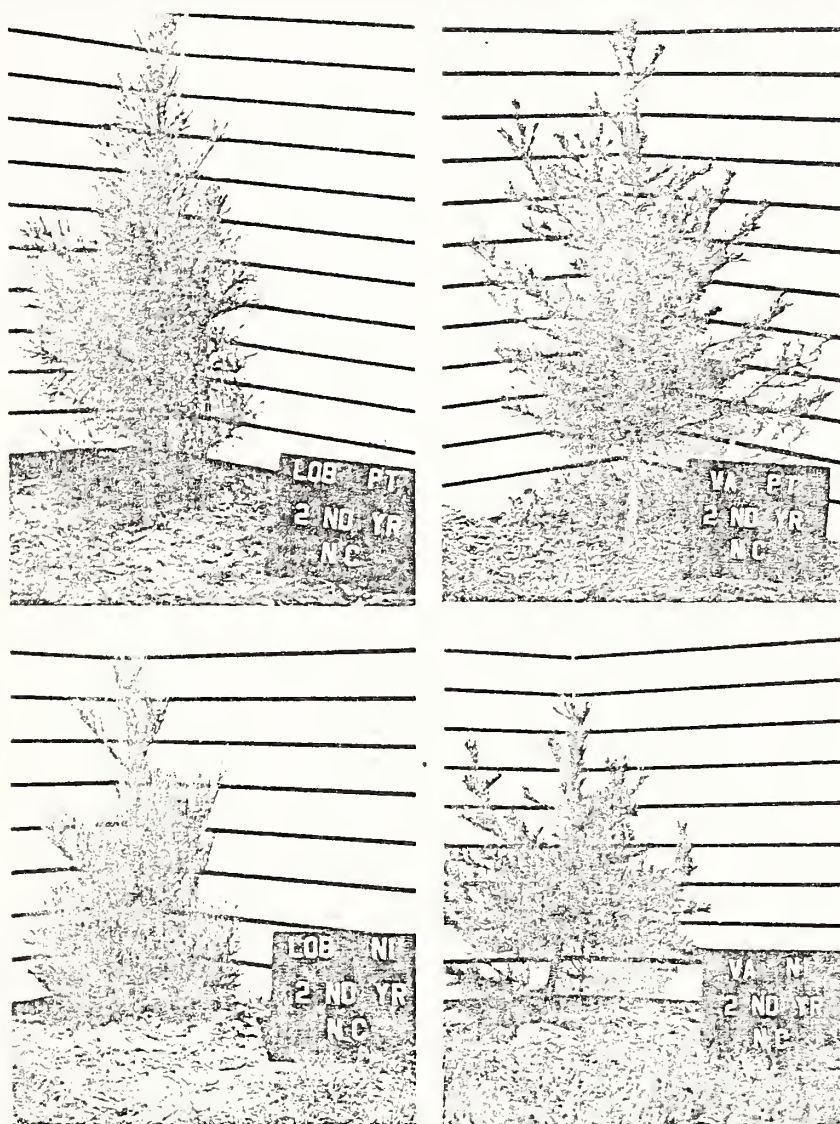
Fig. 2.1. Growth response of pines to specific ectomycorrhizae. Upper left and right photographs are loblolly and Virginia pine seedlings after two years on a routine reforestation site in North Carolina, USA, that had abundant *Pisolithus tinctorius* mycorrhizae on roots at planting time. Lower left and right photographs are corresponding control seedlings that had abundant naturally occurring *Thelephora terrestris* mycorrhizae on roots at planting time. Horizontal lines in background are spaced 10 cm.

observed on new roots of various seedlings regardless of initial mycorrhizal conditions. Sporophores of *P. tinctorius* were also observed in many *Pisolithus* plots. We obtained fourth year data from these seedlings recently (Marx, unpublished data). The differences in growth rate are either greater after the fourth growing season or are approximately the same as those at the end of the second season.

Krugner (1976) grew seedlings of *P. taeda* in a nursery soil of different fertility inoculated with *P. tinctorius*, *T. terrestris*, or a mixture of the two fungi. As discussed earlier, *Pisolithus* in this study formed more mycorrhizae in fertilized than in non-fertilized soil. Selected seedlings were outplanted on two recently prepared sites in the coastal plain of North Carolina. Only data from the first growing season is available. On one site, excessive weed competition and high populations of indigenous ectomycorrhizal fungi apparently eliminated the effects of the inoculation and fertility treatments from the nursery. The other site was considered poor and seedlings with the greatest amount of *Pisolithus* mycorrhizae had up to 19 per cent better survival, 63 per cent more height growth, and 15 per cent larger stem diameters than seedlings with only *Thelephora* mycorrhiza.

These results indicate that under the temporarily adverse situations caused by tree removal and site preparation of routine reforestation sites, pine seedlings may survive and grow faster if they have abundant mycorrhizae formed by a fungus, such as *P. tinctorius*, which is ecologically adapted to adverse conditions. Apparently, fertilization reduces the adverse situation to such a degree that seedlings with *Thelephora* mycorrhizae can survive and grow as well as those with *Pisolithus*. Our results also show that the more *Pisolithus* mycorrhizae seedlings have on their roots at planting, the more benefit they derive from this specific mycorrhizal association, especially on the poorer reforestation sites. Perhaps the ecological adaptation to poor soil conditions allows *P. tinctorius* a competitive advantage over other ectomycorrhizal fungi for colonization of new feeder roots. On better reforestation sites the other fungi may be more competitive and aggressive than *Pisolithus*. We have found from other field experiments that seedlings with *Thelephora* mycorrhizae in non-stress situations survive as well and grow better than seedlings with *Pisolithus* mycorrhiza. These observations coincide very well with what we think we know about the biological significance of certain ecological adaptability traits of these fungi.

Results from these various field studies show that specific ectomycorrhizal fungi can improve initial field performance of tree seedlings on good and poor sites. Some fungi appear to increase tolerance of the seedlings to extremes in soil environment, whereas others appear to enhance absorption of certain nutrients, such as

phosphorus, from the soil. In all probability, many of these fungi share certain traits which act in concert to increase survival or early growth of tree seedlings. Unfortunately, there is no data to show whether these early growth effects have any influence on the final volume of wood harvested at the end of the normal rotation. Only long-range studies can furnish information of this type.

Conclusion

There is no doubt that a variety of proven methods are available to ensure the development of ectomycorrhizae on forest tree seedlings for the establishment of man-made forests. Certain methods have more advantages than others. Some methods, such as the use of pure mycelial inoculum, have more biological advantages than others, but a great deal more research must be done. There is sufficient information, however, to conclude that pure cultures of certain fungi, such as *Suillus granulatus*, *Rhizopogon luteolus*, and *Pisolithus tinctorius* can be used to assure good survival and growth of tree seedlings on a variety of sites. These represent only the beginning of the practical concept, however. When one considers the millions of hectares of potential exotic forests which should be established in Third World nations, as well as the millions of hectares of former forest lands awaiting artificial regeneration throughout the world, the importance of the selection, propagation, manipulation, and management of superior strains or species of mycorrhizal fungi as a forest management tool is paramount. Research so far has only revealed the tip of the iceberg in regard to potential use in world forestry. There still remains a tremendous reservoir of basic and practical information which must be revealed if these fungi are to be managed and, therefore, fully utilized in forest regeneration.

The introduction of ectomycorrhizal fungi into various parts of the world to establish exotic forests has expanded the geographic range of these fungi throughout the world (Mikola 1969, 1970, 1973) Although many species probably died, numerous fungi are currently thriving in areas far distant from their original habitat. These fungi, either individually or as a group, have a tremendous capacity to adapt to different environments. Once techniques have been perfected for use of pure cultures and adequate quantities of inoculum are available, the specific fungi should be tested on tree seedlings over a wide range of environmental conditions encountered in forestation throughout the world. There is not sufficient information available in the world literature on the use of a specific fungus to even remotely suggest where it can or cannot benefit a specific tree species in a given locality. Even though the effect of a given

fungus may only be temporary, its short term influence may make the difference between initial success or failure of seedling establishment. There are several botanical precedents for this idea of testing an organism over a spectrum of environmental conditions beyond those present in its natural habitat. One such example is *Pinus radiata*. Its natural range is restricted to about 4500 hectares along the coast of California. Since the mid-1800s this tree has been planted successfully throughout the world, including such countries as Australia, New Zealand, Chile, Bolivia, Spain, Ireland, and several African nations. By 1958, over 623 000 hectares had been planted (Scott 1960). In many of these countries it has become the major commercial forest tree. There is little doubt that these forests are established today because foresters took a broad ecological view of the potential range of *Pinus radiata*. Researchers on mycorrhizae should also approach the use of specific ectomycorrhizal fungi in world forestry from a broad ecological view until results from research dictate otherwise. Let us determine the biological and practical significance of a given ectomycorrhizal fungus to forest productivity not by supposition, but by facts obtained by using scientific rules of proof.

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Fiber, Food, Fuel, and
Fungal Symbionts

John L. Ruehle and Donald H. Marx

Fiber, Food, Fuel, and Fungal Symbionts

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Forest and agricultural scientists are struggling to increase yields of food, fuel, and fiber which are essential to human needs and which must be produced on a fixed quantity of land. Largely as a result of research on forest trees, a new technology that attempts to use mycorrhizal fungi for the benefit of man is emerging.

Plant scientists have learned to think of a dual system—the soil and the plant. This thinking should be expanded to include a third component—the mycorrhizal fungi. Mycorrhizae (fungus roots)

phosphorus; and plants grow poorly in areas without adequate mycorrhizal fungi. Results of recent research indicate that the growth of forest trees and certain agricultural plants can be stimulated by inoculating them with mycorrhizal fungi when such plants are growing on soils with low levels of mycorrhizal fungi and essential nutrients (3, 4).

Forest trees require mycorrhizae to survive and grow in the natural forest environment. In modern agriculture, crop plants have been selected and bred to give adequate yields only under luxuri-

Summary. Virtually all plants of economic importance form mycorrhizae. These absorbing organs of higher plants result from a symbiotic union of beneficial soil fungi and feeder roots. In forestry, the manipulation of fungal symbionts ecologically adapted to the planting site can increase survival and growth of forest trees, particularly on adverse sites. Vesicular-arbuscular mycorrhizae, which occur not only on many trees but also on most cultivated crops, are undoubtedly more important to world food crops. Imperatives for mycorrhizal research in forestry and agriculture are (i) the development of mass inoculum of mycorrhizal fungi, (ii) the interdisciplinary coordination with soil management, plant breeding, cultivation practices, and pest control to ensure maximum survival and development of fungal symbionts in the soil, and (iii) the institution of nursery and field tests to determine the circumstances in which mycorrhizae benefit plant growth in forestry and agri-ecosystems.

result from symbiotic colonization of fine roots by beneficial soil fungi. The vast majority of economically important plants form mycorrhizae (1). On the basis of their morphology, these associations are currently divided into two major groups: ectomycorrhizae and endomycorrhizae. Of the two, endomycorrhizae are by far the most common (2), but ectomycorrhizae are formed on some very important families of forest trees.

Mycorrhizae of both groups are increasingly recognized as important contributors to the cycling of soil nutrients. Many soils in the world are deficient in available major nutrients, particularly

ant soil fertility conditions (5). Consequently many plant species are cultivated with minimal numbers of mycorrhizae. If fertilizers were plentiful and relatively cheap, there would be no problem. But with the cost of fertilizer increasing rapidly, we can no longer use these chemicals so lavishly. We must find ways to increase plant efficiency in nutrient uptake. The use of efficient mycorrhizal plants in conjunction with reduced fertilizer applications is a viable alternative.

Since mycorrhizal fungi can colonize a given plant species, it may be practical to improve nutrient absorption by judicious selection and management of the more efficient fungi. Such manipulation is likely to be most valuable on nutrient-deficient soils and should be considered in breeding plant varieties that can tolerate low fertility.

Ectomycorrhizae

Ectomycorrhizae are formed by fungi belonging to the higher Basidiomycetes (mushrooms and puffballs), Ascomycetes (cup fungi and truffles), and Phycomycetes in the family Endogonaceae. The host plants of these fungi are predominantly trees such as pine, hemlock, spruce, fir, oak, birch, beech, eucalyptus, willow, and poplar. Many species of fungi may be involved in the ectomycorrhizal associations of a forest, a single tree species, an individual tree seedling, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhizal root cluster. Whereas a single tree species can be host to numerous species of ectomycorrhizal fungi, most fungal species can also form ectomycorrhizae with numerous tree hosts. Although some fungi are fairly host-specific, others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families.

These fungal symbionts are stimulated by root exudates. Hyphae grow over the surface of feeder roots and form a fungus mantle. Hyphae then develop around root cortical cells, completely replace the middle lamella, and form the Hartig net, which is the distinguishing feature of ectomycorrhizae (Fig. 1).

Research on ectomycorrhizal associations on trees growing in natural environments has revealed that (i) any ectomycorrhizae on tree seedlings are better than none and (ii) some species of ectomycorrhizal fungi under certain environmental conditions are more beneficial than others. Appropriate methods of selecting, propagating, manipulating, and managing the most desirable fungal symbionts can lead to improvement in tree survival and growth on a variety of forest sites.

Practical use of ectomycorrhizal fungi can be of major significance in forest regeneration. Inoculation of planting stock with specific ectomycorrhizal fungi can increase survival and growth of seedlings planted on cutover lands, former treeless areas, and disturbed or adverse sites such as mining spoils.

Most work on inoculation with ectomycorrhizal fungi has been done in nurseries that produce bare-root or "containerized" tree seedlings. Another promising application, however, is inoculation of seed for broadcasting on sites that are too remote or too rough for convenient planting of seedlings.

Most ectomycorrhizal fungi produce sporophores (puffballs or mushrooms)
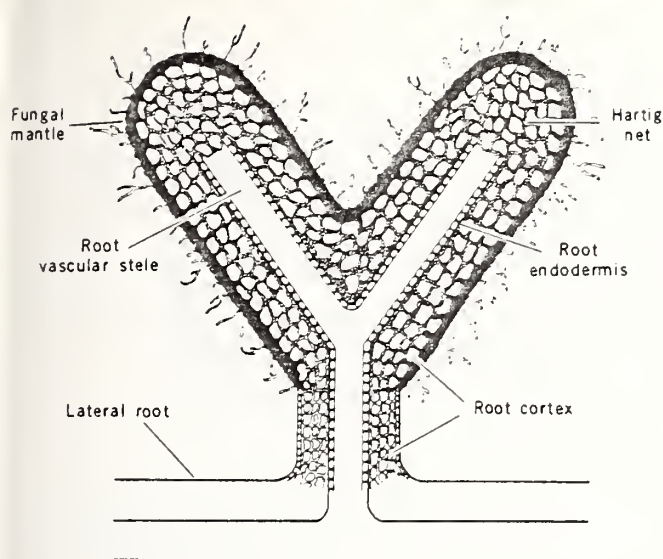
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Fig. 1. Diagram of typical ectomycorrhiza including the Hartig net, fungal mantle, and external hyphae.

containing thousands of spores that can be disseminated great distances by wind, rain, insects, and small mammals. The greater the density of tree stands and the closer the proximity of the tree hosts to the seedling production areas, the greater the chances for rapid natural ectomycorrhizal development on the seedlings. If a nursery is surrounded by stands of forest trees with ectomycorrhizae, fungus spores produced in these stands can be carried by wind and thus can rapidly recolonize fumigated nursery soil. Fumigation before planting is a routine procedure to control pests. Frequently, ectomycorrhizae appear on the roots of seedlings within 6 to 8 weeks after the seedlings emerge from fumigated soil.

Although nursery soils in the southern part of the United States are rarely deficient in ectomycorrhizal fungi, the species that predominate are not always the best for reforestation. The methods used to produce seedlings of pine and oak, whether bare-root or container-grown, favor fungi that are adapted to high soil moisture and fertility. *Thelephora terrestris*, one of the most common symbionts in nurseries in the South, is an example. In the spring, this symbiont releases spores from sporocarps in forests adjacent to nurseries. The spores are windborne to fumigated nursery soil, leached by water a few centimeters, and rapidly colonize newly emerging seedling roots, often precluding colonization by other fungi that produce spores later in the year. Unfortunately, this symbiont is best adapted to fertilized and irrigated nursery soil. Its inability to function on harsh sites results in poor initial host survival and growth.

Many tree species requiring ectomycorrhizae would not reach plantable

size in the nursery if they failed to develop adequate ectomycorrhizae. Ectomycorrhizal deficiencies have been experienced throughout the world in new nurseries established in areas devoid of an adequate airborne inoculum from ectomycorrhizal forests surrounding the nursery. Mycorrhizal deficiencies are also seen in nurseries that use large amounts of soluble fertilizers. Heavy fertilization, especially with nitrogen and phosphorus, changes the biochemical status of seedling roots and reduces their susceptibility to infection by mycorrhizal symbionts (6). In container-seedling nurseries, heavy fertilization to obtain the fastest seedling growth inhibits or eliminates mycorrhizal development.

Ectomycorrhizal fungi have been introduced into nursery soil or container-growing medium in various ways. Early workers applied inoculum in the form of duff, humus, infested soil, crushed sporophores, or excised mycorrhizal roots. Although these methods normally ensure ectomycorrhizal development, they also create problems. The inoculum may lack the most desirable fungi for the tree species and planting sites, it usually contains so much extraneous material that movement is expensive, and it often contains various harmful microorganisms and noxious weeds. In addition, sufficient quantities of sporophores or colonized roots may not be available when needed (7).

Mass production of ectomycorrhizal fungi in pure culture is the best solution, but it is easier said than done. Most fungi grow in pure culture only when very specific conditions are provided, and a great deal of research is required to determine the appropriate conditions. Therefore, this source of inoculum is at present limited to a small number of fungal species.

In Europe, Latin America, and the United States research results with mycelial inoculum of several symbionts have been encouraging. At our institute in Athens, Georgia, inoculation of fumigated nursery soils has improved seedling quality by lowering the cull rate of nursery seedlings and increasing root development and the overall size of seedlings (8). In field trials, pine seedlings inoculated with *Pisolithus tinctorius* in the nursery survive and grow better on adverse and routine reforestation sites than seedlings colonized by naturally occurring fungi (9).

These encouraging results have created sufficient interest in the agrichemical industry to motivate Abbott Laboratories, North Chicago, Illinois, to study ways of producing a dried, vermiculite-peat moss–based inoculum of *P. tinctorius*. This material is being compared to our laboratory-produced inoculum in 46 bare-root and 11 container-tree nurseries throughout the United States. Results indicate that Abbott Laboratories' inoculum can form ectomycorrhizae on several species of pine, oak, spruce, Douglas fir, and hemlock. Thus, if tests with this material continue to be positive, forest nurseries will soon have the means to produce seedlings of these tree species with abundant ectomycorrhizae of a predetermined fungus species.

Endomycorrhizae

Vesicular-arbuscular (VA) endomycorrhizal fungi occur on most food crops throughout the world, but they are ignored by many plant scientists because they have little effect on root morphology and are difficult to detect in roots. Also, these fungi have not yet been grown in pure culture and are not detected in routine soil assays with nutrient media.

The fungi invade the cortex, but not the endodermis or stele, of feeder roots. Colonization does not alter root morphology, and the sheath of fungus mycelium common to ectomycorrhizae is lacking. Under a microscope, VA mycorrhizae are diagnosed by the presence of vesicles (terminal, spherical structures that contain oil droplets) and arbuscules (complex structures formed by repeated dichotomous branching of hyphae) in the cortical cells of differentially stained feeder roots. Mycelia emanate from the infected root to form a loose network in the rhizosphere and adjacent soil (Fig. 2).

The VA mycorrhizae are formed by certain fungal species of the family
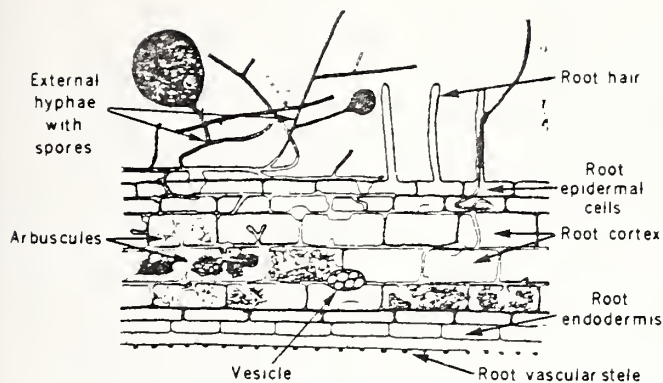
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Fig. 2 (left). Diagram of typical endomycorrhiza including arbuscules, vesicles, and external hyphae with spores. [Drawing by F. E. Sanders with permission from Academic Press, London]   Fig. 3 (right). Growth response of 6-month-old sweetgum seedling colonized with endomycorrhizal fungi. The pair on the left is colonized with *Glomus* spp.; the pair on right is nonmycorrhizal.
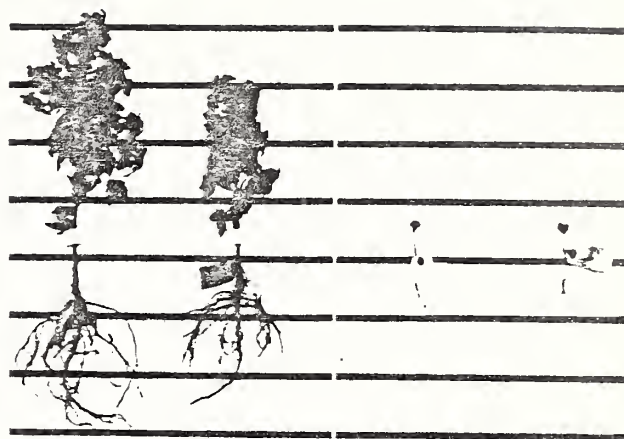
Liquidambar styraciflua
0 ppm
10 November 1977

Endogonaceae. These symbiotic fungi produce large, globose to ovoid spores. The spores are grouped in sporocarps or appear singly in the soil or in plant roots. These sporocarps or spores, hypogenous beneath leaf litter or in the mineral soil, occasionally become airborne during dust storms in semiarid regions. These fungi are more commonly spread by growing from feeder root to feeder root and, at times, are disseminated by moving water, soil, insects, and animals.

The importance of VA endomycorrhizae to phosphate nutrition is indicated by recent research (3). The VA endomycorrhizae significantly increased growth of plants—by several hundred percent in some instances—on soils deficient in readily available phosphate. The main effect of plant response to these symbionts is increased efficiency of nutrient uptake. Thus, if plants are colonized with appropriate VA endomycorrhizal fungi, the estimate of production potential of a soil and its fertilizer requirement might change radically. If methods of producing large quantities of inocula are devised, large-scale inoculation in agriculture and forestry to increase plant yield may become feasible.

Relationships Between Symbiont and Host

Ion uptake in plants is governed by the absorbing capacity of the root and the movement of ions to the root. Plant uptake of highly mobile ions—such as nitrate, sulfate, and potassium—are largely determined by the absorbing capacity of roots. Relatively low concentrations of feeder roots through the soil profile may be adequate for uptake of such ions

in soil. However, for relatively immobile ions, such as phosphate, zinc, copper, molybdenum, and sometimes ammonium, movement to the root is a limiting factor. In these cases, the distribution of root hairs and mycorrhizal fungi in the soil may determine the rate of ion uptake (10).

Plant species differ in their production of roots and root hairs (11). Many tree species have few fine feeder roots and few or no root hairs. They have far less direct contact with the soil and soil solution than grains and grasses, which have high root volumes with many fine roots and root hairs. One of the main benefits of VA endomycorrhizae is hyphal growth into soil. These structures absorb ions and transport them back to the higher plants. Therefore, plants with few feeder roots and root hairs should benefit more from mycorrhizal infection than those with numerous feeder roots (11).

Mycorrhizae accumulate more soluble phosphorus from soil than nonmycorrhizal roots because hyphae emanating from mycorrhizae extend beyond the zone of phosphorus depletion that develops adjacent to the root epidermis (12). Also, VA endomycorrhizae improve uptake of other nonmobile ions; in zinc-deficient soil in California, for example, peach seedlings with VA endomycorrhizae were able to take up much more zinc and to grow larger than nonmycorrhizal seedlings (13).

Mycorrhizal fungi may influence processes other than ion uptake (14). Some mycorrhizal fungi can grow at much lower water potential than higher plants. Hyphae extending into the soil, therefore, can increase water movement to the roots (15). Plants with few root hairs and feeder roots probably benefit from symbiotic infection when growing in

sandy or semiarid soils. Thus, instead of irrigating to accommodate a crop, we might alter plants by genetic and mycorrhizal manipulation and tailor the crop to the existing droughty environment. In forests and grasslands, mycorrhizal associations help to conserve and cycle nutrients. Fungal hyphae readily penetrate litter and decomposing organic matter and can spatially compete with other soil microorganisms for organic and inorganic nutrients far more efficiently than nonmycorrhizal plant roots. Root destruction by soil-borne pathogens in nutrient-deficient soils will often lead to reduced plant yield. Active mycorrhizae and hyphal growth into soil from roots in other parts of the root system not infected by pathogens can compensate for a certain degree of root loss (3). In most instances the mycorrhizae are resistant to attack by fungal pathogens (4).

The size and effectiveness of the native VA endomycorrhizal fungus population in different soils varies. If the population is small, mycorrhizal infection may be sparse in the early stages of plant growth when the need for phosphate is the greatest, and the infection can inhibit seedling establishment and subsequent growth. Furthermore, the species or strains of endophyte present may not be the most efficient. Many naturally occurring species have no marked effect on growth of certain host plants. Further research is needed to ensure selection of highly efficient fungi for particular soils and crops and to develop methods for their introduction and maintenance.

The indigenous symbiont population can also be strongly affected by certain agricultural practices. For example, the use of soil fumigants or other pesticides to kill plant pathogens and nematodes also reduces populations of mycorrhizal

fungi. Their populations may also decline in the absence of suitable host plants during fallow periods or during prolonged growth of nonhost plants.

In England, soil from a field of heavily mycorrhizal barley was used to inoculate a phosphorus-deficient soil in which the population of endomycorrhizal spores was low. The colonized soil was incorporated into the planting furrows, and the yield of potatoes was increased by nearly 20 percent with no additional phosphorus fertilizer (16). In other field experiments in Pakistan, transplants of corn, wheat, and barley that had been inoculated with VA endomycorrhizal fungi grew faster than uninoculated controls in a very infertile soil (17). In the same experiment, the growth response to mycorrhizae was nullified when high rates of phosphorus fertilizer were added.

Large growth responses to inoculation with VA mycorrhizae are more likely to occur in tropical than in temperate soils. Many of the arable soils of the tropics are highly leached Oxisols and Ultisols and are low in bases and relatively acid; these soils contain high amounts of exchangeable aluminum. Tropical soils are commonly deficient in phosphorus and other essential elements and they tend to immobilize added phosphorus. Rock phosphate is cheaper and more plentiful than superphosphates in tropical countries, but it is also less soluble. Particularly important, therefore, is the discovery that the interaction of rock phosphate with artificially introduced VA endomycorrhizae increases plant yields in tropical soils (3).

When the soil is fumigated to control disease, plants that depend heavily on VA mycorrhizae grow poorly unless the soil is inoculated with suitable fungi or high rates of phosphorus fertilizer are added. For example, some citrus cultivars are strongly dependent on mycorrhizae. They are severely stunted in fumigated nursery soil unless several hundred kilograms of phosphorus per hectare is added (12). Stunting can also be corrected by inoculating the soil with appropriate VA endomycorrhizal fungi. A similar relation has been observed with sweetgum trees in Georgia (18) (Fig. 3).

Pure culture techniques for mass producing inoculum of endomycorrhizal fungi are lacking. However, on a limited scale colonized soil or plant roots have been successfully used as the inoculum. Crops normally transplanted to the field could be grown in containers with selected fungi and their pretransplant mycorrhizal status monitored. Soil or sievings from pot cultures (an inoculum mixture of spores, hyphae, and infected root pieces) can be incorporated into the planting furrows. This technique has been successful in experiments on fumigated soils that contained no indigenous endomycorrhizal fungi (18). The VA mycorrhizal fungus populations can be increased by growing a heavily mycorrhizal crop in a field plot and then using the topsoil as crude inoculum (12). In this method, care must be taken to prevent contamination with pathogens and weeds.

If inoculum of indigenous fungal symbionts is needed, soil containing them can be fumigated to remove harmful microorganisms and noxious weeds and then seeded with a suitable cover crop to increase the residual inoculum that survived fumigation. The practical, large-scale use of specific symbionts must be produced in pot cultures in large quantities, readily transported to the field, and easily introduced into the seedling root zone. Seed inoculation might be tried as an alternative because it would require smaller amounts of inoculum and virtually no change in planting procedures (19).

Conclusion

Major increases in world production of food and fiber over the next few decades can result from a better understanding of the organisms and processes of the rhizosphere. The management systems that produce high yields in developed nations are often not directly transferable to developing nations. Furthermore, the environmental effects and energy costs of some of these systems are being questioned in the United States. Some treatments, such as fertilizer applications, have been formulated solely on the basis of the response of the crop plant. Until we understand the complex chemistry, physics, and biology of the rhizosphere, there remains no way of knowing whether other approaches would be cheaper or more effective.

In our work with mycorrhizal symbiosis on forest trees, results are very promising, and we have really just begun. When a mycorrhizal symbiont that is ecologically adapted to the planting site is used, seedling survival and growth can be improved on a variety of sites. When one considers the millions of hectares of potential exotic forests that might be established in Third World nations, as well as the millions of hectares of former forest lands awaiting artificial regeneration in the developed world, the importance of such treatment becomes apparent.

We have known about possible benefits of ectomycorrhizae in forestry for some time, but only in the last few years has the significance of endomycorrhizae begun to be appreciated. These structures are common not only in many species of forest trees, but also in most crop plants. We cannot help but believe that mankind will benefit from the manipulation of these symbiotic associations.

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USE OF SPECIFIC MYCORRHIZAL FUNGI ON TREE ROOTS
FOR FORESTATION OF DISTURBED LANDS

Donald H. Marx[1]

Abstract.--The significance of ecologically adapted
ectomycorrhizal fungi such as Pisolithus tinctorius to
survival and growth of pines and other tree species has
practical importance for forestation of disturbed lands.
Data from studies on strip-mined coal spoils in Virginia
and Kentucky, strip-mined kaolin spoils in Georgia, and
eroded sites in Copperhill, Tennessee, show that pines
whose root systems are tailored in the nursery with Piso-
lithus ectomycorrhizae prior to planting survive and grow
significantly better than trees with species of other
fungal symbionts. Performance of hardwood tree species,
such as sycamore, on kaolin spoils also has been improved
by specific endomycorrhizal fungi. Manipulation of mycor-
rhizal fungi on tree roots has great potential in reclaim-
ing drastically disturbed lands as well as in routine re-
forestation efforts.

Additional keywords: Soil infestation with mycorrhizal
fungi, Thelephora terrestris, Glomus mosseae, ecologically
adapted fungal symbionts.

Microorganisms are present in great numbers near the feeder roots
of plants, and they play vital roles in numerous physiological process-
es. These dynamic microbial processes include saprophytism, pathogeni-
city, and symbiosis. The most widespread symbiosis of plants is the
mycorrhizal association involving root-inhabiting fungi and plant feeder
roots. The prevalence of mycorrhizal associations on plants is so com-
mon under natural soil conditions that a nonmycorrhizal plant is the
exception. Few plants, such as sedges, crucifers and certain aquatics,
fail to form mycorrhizae. Other plants, especially those of major eco-
nomic importance to man, such as forest trees and agronomic crops, form
abundant mycorrhizae on their roots.

CLASSES OF MYCORRHIZAE

There are three kinds of mycorrhizae: ectomycorrhizae, endomycor-
rhizae and ectendomycorrhizae. Ectomycorrhizae occur naturally on many
important forest tree species around the world. All members of the
gymnosperm family Pinaceae (pine, spruce, fir, larch, hemlock, etc.) as
well as certain angiosperms (willow, poplar, aspen, walnut, hickory,
pecan, oak, beech, eucalypt, etc.) are ectomycorrhizal. Some species
can be either ectomycorrhizal or endomycorrhizal depending on soil con-

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ditions. Ectomycorrhizal infection is initiated from spores or hyphae (propagules) of the fungal symbionts inhabiting the rhizosphere of the feeder roots. Propagules are stimulated by root exudates and grow vegetatively over the feeder root surface, thus forming a fungus mantle. Following mantle development, hyphae develop intercellularly around root cortical cells and form the Hartig net, which may completely replace the middle lamellae between the cortex cells. The Hartig net is the main distinguishing feature of ectomycorrhizae. Externally, ectomycorrhizae may appear as simple unforked roots, bifurcate roots, multiforked (coralloid) roots, nodular-like roots, or in other configurations. The color of an ectomycorrhiza is apparently determined by the color of the hyphae of the fungal symbiont and may be brown, black, white, red, yellow, or blends of these colors. Individual hypha, strands of hyphae, or rhizomorphs may radiate from the fungus mantles into the soil and to the base of the fruit bodies of the fungi.

Most fungi which form ectomycorrhizae with forest trees are Basidiomycetes that produce mushrooms or puffballs (fruit bodies). Certain Ascomycetes however, such as truffles, also form mycorrhizae. Over 2100 species of ectomycorrhizal fungi are estimated to exist on trees in North America. The fruit bodies of these fungi produce millions of spores that are widely disseminated by wind and water. Most ectomycorrhizal fungi are dependent on their hosts for essential carbohydrates, amino acids, vitamins, etc., necessary to complete their life cycles. Ectomycorrhizal development, therefore, is usually a prerequisite for fruit body production by these fungi; not all fungi which form mushrooms and puffballs, however, are ectomycorrhizal. Many of these fungi are saprophytes that play an important role in the decomposition of organic matter and the mineral cycle in forest soils. Certain other species are pathogenic to trees.

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest, a single tree species, an individual tree, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza. Even as a single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots, a single fungus can enter into ectomycorrhizal association with numerous tree species. Some fungi are apparently host-specific; others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families.

Ectomycorrhizal fungi aid the growth and development of trees. For some trees, such as Pinus, they are indispensable for growth under natural field conditions. Trees with abundant ectomycorrhizae have a much larger, physiologically active, root-fungus area for nutrient and water absorption than do trees with few mycorrhizae. This increase in surface area comes both from the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae of the fungus symbionts from the ectomycorrhizae into the soil. These extramatrical hyphae function as additional nutrient and water-absorbing entities

and assure maximum nutrient capture from the soil by the host. Ecto-
mycorrhizae are able to absorb and accumulate nitrogen, phosphorus,
potassium, and calcium in the fungus mantles more rapidly, and for
longer periods of time, than nonmycorrhizal feeder roots. Ectomycor-
rhizal fungi help break down certain complex minerals and organic sub-
stances in the soil and transmit nutrients from these materials to the
tree. Ectomycorrhizae also appear to increase the tolerance of trees
to drought, high soil temperatures, soil toxins (organic and inorganic),
and extremes of soil pH caused by high levels of sulfur or aluminum.
Ectomycorrhizae function as biological deterrents to infection of feeder
roots by root pathogens, such as species of Pythium and Phytophthora.
Induced hormone relationships in ectomycorrhizae by fungal symbionts
extend the longevity (length of physiological activity) of these roots
as compared to nonmycorrhizal roots.

Endomycorrhizae are formed on most of the economically important
agronomic and forage crops, as well as fruit and nut trees (peach,
citrus, apple, plum, cherry, almond, etc.). Important forest trees
(maples, elms, gums, ash, sycamore, alder, and dogwood) and other tree
species not forming ectomycorrhizae normally form endomycorrhizae.
Some of these trees have the capacity to form both endo- and ectomycor-
rhizae. Endomycorrhizal fungi form a loose network of hyphae on feeder
root surfaces and do not develop the dense fungus mantle of ectomycor-
rhizae. These fungi often form large, conspicuous, thick-walled spores
both on the root surfaces and in the rhizosphere, and sometimes in
feeder root tissues. Hyphae of the endomycorrhizal fungi penetrate the
epidermal cell walls and progress into the cortical cells of the root.
These infective hyphae may develop specialized absorbing or nutrient-
exchanging structures (haustoria) called arbuscules in the cortical
cells. Thin-walled, spherical-to-ovate vesicles may also be produced
in the cortex cells by these fungi. The term "vesicular-arbuscular"
mycorrhizae has been coined to denote this type of endomycorrhizae.
As in ectomycorrhizae, endomycorrhizal fungus infection rarely occurs
in meristematic or vascular tissues. Unlike ectomycorrhizae, endomy-
corrhizal infection does not cause major morphological changes in roots.

The fungi which form endomycorrhizae with trees are mainly Phyco-
mycetes. They do not produce large, above-ground fruit bodies or wind-
disseminated spores as do most ectomycorrhizal fungi, but some of them
produce large zygospores and chlamydospores on or in roots. Some species
also produce on roots large sporocarps (5-10 mm diameter) containing
many spores. These fungi spread through the soil by root contact, moving
water, insects, or animals. Many endomycorrhizal fungi of trees belong
to the family Endogonaceae. Several genera and species have been identi-
fied; many more undoubtedly exist (Gerdemann and Trappe 1974). They are
so widespread that it is difficult to find natural soils anywhere in the
world that do not contain them. Spores of these fungi are able to sur-
vive in soil for years without a host. Based on the limited research
done on endomycorrhizal fungi, most species appear to have very broad
host ranges. For example, Glomus mosseae (= Endogone mosseae) will form

endomycorrhizae with cotton, corn, pepper, soybean, and sorghum, as well as sycamore, sweetgum, citrus, peach, black locust, green ash, white ash, black cherry, and box elder, and undoubtedly can infect numerous other plant species.

Very little research has been conducted to determine the value of endomycorrhizae to forest trees. Available research results indicate that endomycorrhizal roots absorb nutrients better than nonmycorrhizal roots. This applies especially to the absorption and utilization of phosphorus. The extensive network of hyphae of these fungi growing from endomycorrhizae capture nutrients from large volumes of soil that are rarely explored by nonmycorrhizal roots. It is not known for certain if some trees are dependent on endomycorrhizae for growth like Pinus species are on ectomycorrhizae. Recent evidence obtained from research at the Athens, Georgia, Laboratory, U. S. Forest Service, however, indicates that sweetgum and other important forest tree hardwoods may need endomycorrhizae in order to become established and to grow normally in nurseries.

Ectendomycorrhizae, another class of mycorrhizae, have the features of both ecto- and endomycorrhizae. Ectendomycorrhizae have a limited distribution in forest soils and are found primarily on roots of normally ectomycorrhizal trees. Very little is know about the species of fungi involved or their importance to tree growth because little research has been done on them.

FACTORS AFFECTING MYCORRHIZAL DEVELOPMENT

Many factors affect mycorrhizal development. In discussing these factors it is necessary to separate those which affect the tree from those which affect the fungal symbionts. Generally, any soil or above-ground condition which influences root growth also influences mycorrhizal development. The main factors influencing susceptibility of tree roots to mycorrhizal infection appear to be photosynthetic potential and soil fertility. High light intensity and low to moderate soil fertility enhance mycorrhizal development; the other extremes of these conditions (light intensity below 20% of full sunlight and excessively high soil fertility) reduce, or may even prevent, mycorrhizal development. Light intensity and fertility appear to influence either the biochemical status of feeder roots, such as controlling levels of sugars, or the synthesis of new feeder roots, both of which are prerequisites to symbiotic infection. Roots growing rapidly because of high soil fertility may actually outgrow their fungal symbionts. The factors which affect the fungal symbionts directly are those which regulate survival or growth of infective propagules of the symbionts on the roots. Extremes of soil temperatures, pH, moisture, etc., and presence of antagonistic soil microorganisms can affect the survival of symbionts and thereby influence the mycorrhizal potential of the soil. Research has not shown that mycorrhizal fungi can grow and reproduce in soil without a symbiotic association with plant roots. These fungi are capable, however, of surviving in a dormant condition for extended periods of time

without a plant-host association.

Several excellent texts have been published which discuss the different aspects of mycorrhizae (Harley 1969, Marks and Kozlowski 1973, Hacskaylo 1971).

MYCORRHIZAE AND AFFORESTATION PRACTICES

Over 3000 papers have been published on mycorrhizae, and most of these relate to forest trees (Hacskaylo and Tompkins 1973). Much of this research has been basic and has broadened our understanding of the complexity of mycorrhizae in forest soil ecosystems. Various studies have shown that many forest trees cannot grow without ectomy-corrhizae. This means that certain trees, such as Pinus, have an obli-gate requirement for ectomycorrhizae. This basic information implies that any kind of ectomycorrhizae is better than no ectomycorrhizae at all, especially on pines. This point has practical significance to afforestation programs with normally ectomycorrhizal trees in areas of the world where their symbiotic fungi do not occur naturally. Mikola (1969) has demonstrated that a parallel introduction of the essential ectomycorrhizal fungi is necessary if afforestation with these trees (Pinus in particular) is to succeed. There are many areas of the world where indigenous ectomycorrhizal trees and their symbiotic fungi do not occur naturally. In these diverse areas, i.e., the high Andes of Peru (Marx 1976a), Australia (Bowen et al. 1973), Asia (Oliveros 1932), subalpine areas of Austria (Moser 1963), Puerto Rico (Vozzo and Hacskaylo 1972), Africa (Gibson 1963), former agricultural soils of Poland (Dominik 1961), oak shelterbelts on the steppes of Russia (Imshenetskii 1967), and former treeless areas of the United States (Hatch 1937), forestation attempts were either total or near failures until ectomycorrhizae occurred on tree roots. Symbiotic root infection was insured either by introducing soil containing ectomycorrhizal fungi or by manipulating soil containing low levels of indigenous symbiotic fungi to encourage ectomycorrhizal development. Introduction of pure cultures of specific ectomycorrhizal fungi has usually failed.

Natural soils of the world which have supported vegetation in the past have not been reported void of endomycorrhizal fungi. The broad host range of most species of endomycorrhizal fungi normally ensures endomycorrhizal development on desirable plants. Failure in establish-ment of normally endomycorrhizal trees because of a deficiency of endo-mycorrhizal infection in normal soils has not been reported. However, slow early growth of certain hardwood species may be the result of inadequate amounts of endomycorrhizae at planting time.

MYCORRHIZAE AND REVEGETATION OF DISTURBED SURFACE AREAS

The status of the mycorrhizal potential of disturbed surface areas is of concern to us. With the exception of a few reports, very little is known about the mycorrhizal fungi on plants growing on strip-mined

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spoils, borrow pits, severely eroded lands, and other disturbed sites.
There are several questions which must be answered in relation to
forestation of such areas. Can indigenous and recolonizing mycorrhizal
fungi survive the soil conditions of these sites? Are mycorrhizae
essential to all plants that occur naturally or are planted on these
areas? Is there a significant potential for ecological selection among
the vast numbers of species of mycorrhizal fungi to assure that adapt-
able ones will maintain themselves on the roots of plants in these dis-
turbed sites?

Ectomycorrhizal associations.--Only limited research has been done
on ectomycorrhizal associations of trees on strip-mined lands and mining
wastes. In 1966, Schramm published a classic piece of work on plant
colonization of anthracite wastes in Pennsylvania. He concluded that
early ectomycorrhizal development was essential on this waste material
for seedling establishment of Betula lenta, B. populifolia, Pinus
rigida, P. virginiana, Populus tremuloides, Quercus rubra, and Q.
velutina. The only generally successful original plant colonists of
this bare and predominantly nitrogen-deficient waste were either
nitrogen-fixing plants or certain ectomycorrhizal tree species. Schramm
suggested that due to their year-round effectiveness, evergreen trees
(pines) should receive special attention and furnished strong evidence
to support his conclusions. Volunteer seedlings or seedlings from planted
seed of these tree species that did not have ectomycorrhizae became chlo-
rotic and soon died. The majority of surviving seedlings, especially
those groiwng well, were heavily ectomycorrhizal. The main basidiomy-
cetes observed by Schramm associated with these seedlings of the above
tree species were Inocybe lacera, Thelephora terrestris, Pisolithus
tinctorius, Amanita rubescens, and Scleroderma aurantium, all of which
form ectomycorrhizae on various trees (Trappe 1962). His observations
add additional evidence to that already discussed on the need of these
tree species for ectomycorrhizal associations.

Schramm (1966) traced the extensively developed mycelial strands
of P. tinctorius from ectomycorrhizae through large waste volumes to
the base of the fungus basidiocarp. These large, brilliant gold-yellow
mycelial strands were easily traced with the naked eye through the con-
trasting dark anthracite wastes. Some strands were traced through
waste material as far as 15 feet from the seedlings to the basidiocarp.
The ectomycorrhizae he associated with Pisolithus were also gold-yellow
in color and prolifically branched. Seedlings with P. tinctorius ecto-
mycorrhizae were the most vigorously growing seedlings and, in most
cases, these ectomycorrhizae were the first detected on seedling roots.
The other species of ectomycorrhizal fungi appeared on roots and pro-
duced basidiocarps primarily after litter had accumulated under the
older seedlings. These observations tentatively confirmed that P.
tinctorius was the fungal symbiont forming the gold-yellow ectomycor-
rhizae on these tree species. Earlier, Bryan and Zak (1961) formed
ectomycorrhizae with P. tinctorius on Pinus echinata seedlings in
aseptic culture. This pine species, however, was not encountered by
Schramm in Pennsylvania.

Schramm's work strongly suggests that a few ectomycorrhizal fungi may be capable of ecologically adapting to soil conditions on the anthracite wastes. High soil temperatures found on coal wastes and spoils could be a limiting factor in the establishment of specific mycorrhizal fungi. Marx and coworkers (1970) found the P. tinctorius formed more ectomycorrhizae on P. taeda seedlings at a constant soil temperature of 34°C than at 14, 19, 24, or 29°C. In this study, Thelephora terrestris did not form ectomycorrhizae at soil temperatures of 34°C and formed more between 14 and 24°C than at 29°C. T. terrestris, one of the major ectomycorrhizal fungi on pine seedlings in nurseries throughout the United States, is also found on pines planted on coal spoils. It was on the seedling roots from the nursery prior to planting on the spoil. In a later study, Marx and Bryan (1971) found that P. taeda seedlings with Pisolithus ectomycorrhizae survived and grew as well at 40°C as they did at 24°C. Seedlings with Thelephora ectomycorrhizae or without ectomycorrhizae survived poorly and did not grow at 40°C. This temperature tolerance may explain why Pisolithus is the primary symbiont on young volunteer seedlings growing on anthracite wastes. Schramm (1966) recorded soil temperatures between 35°C and 65°C in wastes at a depth of 6 to 7 cm. Perhaps Pisolithus was dominant because high soil temperatures restricted earlier establishment of the other symbiotic fungi.

Prompted by the above reports, we made extensive examinations of various strip-mined spoils in the East. We found Pisolithus basidiocarps and its characteristic gold-yellow ectomycorrhizae and mycelial strands to be the predominant, if not the only, ectomycorrhizal fungus on roots of Pinus virginiana, P. taeda, P. resinosa, and several spp. of Betula and Quercus on coal spoils in Indiana, Pennsylvania, Ohio, West Virginia, Virginia, Kentucky, Tennessee, and Alabama, and P. echinata and P. taeda on strip-mined kaolin spoils in Georgia. Some of these spoils had a soil reaction as low as pH 2.9, although most had a reaction between pH 3.5 and 5.5. Basidiocarps of Pisolithus have also been reported on coal spoils under B. lenta, B. pendula, B. populifolia, Populus grandidenta, P. tremuloides, and Salix humilis in West Germany (Meyer 1968), Pinus banksiana in Missouri (Lampky and Peterson 1963), and Pinus spp. in Indiana and Tennessee (Hile and Hennen 1969).

The following is an example of the prevalence of Pisolithus on disturbed lands. On a kaolin spoil in central Georgia, basidiospores of Pisolithus were collected from basidiocarps under loblolly pine. In less than 12 man-hours, over 12 kilograms of spores were extracted from basidiocarps. There are approximately 1.1 billion spores per gram. Just to illustrate the inoculum potential of Pisolithus on this site, this one collection contained approximately 12.5 trillion basidiospores. Basidiospores are functional inoculum for ectomycorrhizal development on pines (Marx 1976b, Marx and others 1976).

Based on Schramm's observations and other reports, it seems that Pisolithus may be more beneficial to the establishment of ectomycorrhizal

trees on strip-mined and other disturbed lands than are other species
of ectomycorrhizal fungi. Making the assumption that Pisolithus ecto-
mycorrhizae are instrumental in tree establishment and maintenance on
strip-mined spoils, we began to develop techniques to "tailor" seedlings
in the nursery with Pisolithus ectomycorrhizae. The working premises
were simple -- Why wait for natural inoculum to establish Pisolithus
ectomycorrhizae on colonizing trees? Could tree survival and growth be
improved by having Pisolithus ectomycorrhizae develop on the seedlings
in the nursery prior to planting on the disturbed lands? Recently,
techniques were perfected for this "tree-tailoring" concept (Marx and
Bryan 1975) that have been effective on several species of pine in con-
ventional tree nurseries (Marx and others 1976c). Briefly, the techni-
ques involve the production of a pure culture, vegetative mycelial
inoculum of P. tinctorius in a vermiculite-peat moss-nutrient substrate
or basidiospores mixed with a physical carrier, such as moist vermicu-
lite. The inocula are used to infest fumigated nursery soil and fur-
ther modifications of standard nursery practices are not necessary.
Effective soil fumigation (methyl bromide) shortly before soil infesta-
tion and the maintenance of reasonable levels of soil fertility through
the growing season appear to be the only prerequisites for successfully
"tailoring" pine seedlings with Pisolithus ectomycorrhizae. These
techniques also have been used successfully with other ectomycorrhizal
fungi, such as Thelephora terrestris and Cenococcum graniforme.

  The introduction of Pisolithus into nursery soils has significantly
improved pine seedling quality in comparison to standard grown seedlings
with Thelephora ectomycorrhizae. Growth increases in the nursery be-
tween 100 and 150 percent after one growing season have been demonstrated
with seedlings of P. taeda, P. strobus, and P. virginiana following soil
infestation and ectomycorrhizal development by Pisolithus (Marx and others
1976c). After a few more years of research, we are confident that ecto-
mycorrhizal deficiencies in nurseries encompassing many acres can be
corrected by using pure cultures of highly beneficial symbionts. In the
past these ectomycorrhizal deficiencies were corrected by the addition
of forest litter and humus to the nursery soil. This practice sometimes
failed to produce adequate mycorrhizal infection, and in some instances
inadvertently introduced a considerable number of pests such as weeds
and disease-causing organisms into nurseries.

    The practicability of introducing Pisolithus and other ectomycor-
rhizal fungi into the near-sterile root substrate of containerized
seedlings is also being examined. Preliminary information suggests
that growth in containers and field performance of these seedlings can
be improved with specific ectomycorrhizae (Marx and Barnett 1974).
Since it is anticipated that in the near future container stock will
account for as much as 20 percent of the seedlings used in reforestation
in North America, use of specific mycorrhizal fungi could be as rele-
vant to containerized tree stock as it is to standard nursery-grown
seedlings. Container stock also has promise for reforesting strip-mined
lands.

Since techniques to "tailor" tree seedlings with Pisolithus ectomycorrhizae are new, only limited data on seedling performance on disturbed lands are available. Since the winter of 1973, our Research Work Unit in Athens and our cooperators have outplanted nearly 25,000 tree seedlings with specific ectomycorrhizae or endomycorrhizae on a variety of disturbed surface areas. Some of the results are as follows:

Coal spoils in Kentucky.--In cooperation with the Reclamation Project, U.S. Forest Service, Berea, Kentucky, Virginia and red pine seedlings were grown for 5 months in soil infested with either Pisolithus or Thelephora in a mycorrhizal growth room in Athens, Georgia. These seedlings were not dormant at time of planting (April 1973) and subsequently many were killed by late frost. Conventional nursery grown Virginia and red pine seedlings were also planted. Roots of half these seedlings were dusted with dry basidiospores of Pisolithus and the other half remained as controls. A randomized block design was used for outplanting. Three blocks containing a row of 20 seedlings per treatment were utilized. Survival and growth differences between ectomycorrhizal treatments were apparent two years after planting (Table 1). With the exception of the conventional nursery grown Virginia pine seedlings, Pisolithus treatment significantly improved seedling survival. Growth was also improved by Pisolithus in most instances. The incidence of Pisolithus ectomycorrhizae after two years indicates its persistence and its ability to spread via basidiospores and colonize roots of seedlings which had Thelephora ectomycorrhizae at time of planting.

A second planting was installed in Kentucky on a very toxic coal spoil that previously had been unsuccessfully planted with pine several times. Virginia pine seedlings were grown with either Pisolithus or Thelephora ectomycorrhizae in our experimental nursery in Athens (Marx and Bryan 1975). Our nursery is maintained like a conventional tree nursery in regard to soil fumigation, irrigation, pesticide use, etc. The coal spoil planting was a complete randomized plot design, with 4 plots per mycorrhizal condition with each plot containing 40 test seedlings. The results after two years were dramatic (Table 2). Only two of the 160 seedlings with Thelephora ectomycorrhizae survived, whereas 78 of the 160 seedlings with Pisolithus ectomycorrhizae survived. More significant is the fact that Pisolithus seedlings grew extremely well while the two Thelephora seedlings were no larger after two years on site than they were at planting. Basidiocarp production by Pisolithus in the Pisolithus plots was prolific.

Coal spoils in Virginia.--In cooperation with Joel Artman, Virginia Division of Forestry, Charlottesville, loblolly pine seedlings were grown with Pisolithus or Thelephora ectomycorrhizae in the Athens nursery and outplanted on three coal spoil sites in Virginia in 1973. Unfortunately, one planting was totally destroyed by an accidental seeding with lespedeza and a high rate of fertilizer application. Certain blocks of the other two plantings were destroyed by trail bikes, cattle grazing or flooding. Therefore, second year data obtained from the remnants of these plots have no statistical basis, but do show trends (Table 3). In

Table 1.--Survival and growth of Virginia and red pine seedlings with different ectomycorrhizae after two years on a coal spoil in Laurel County, Kentucky[1]

Pine species	Ectomycorrhizal condition at planting	Percent Survival	Height (cm)	Stem Dia. (cm)	$(Stem\ dia.)^2$ X height (volume)	Percent Pisolithus[2]
Virginia[3]	Pisolithus	28*	37.8*	1.14*	49.1*	70*
	Thelephora	15	18.1	0.72	9.4	8
Red[3]	Pisolithus	22*	8.5	0.43	1.6	82*
	Thelephora	7	7.0	0.41	1.2	10
Virginia[4]	SNS + spores	25	41.2	1.30	69.6*	85*
	SNS	32	37.6	1.14	48.9	40
Red[4]	SNS + spores	55*	18.1	0.74	9.9*	65*
	SNS	40	17.3	0.57	5.6	15

[1] Spoil analyses were pH 3.0, 0.14% total N, 0.05% total S, and 2.7% organic matter, with 13, <48, 378, 114 and 29 lbs/acre of P, K, Ca, Mg and NO_3, respectively.

[2] Percent of surviving seedlings with Pisolithus ectomycorrhizae after two years on site.

[3] Seedlings grown in Athens growth room for five months. Due to lack of dormancy at planting many seedlings died following late frosts.

[4] Standard nursery stock seedlings with or without Pisolithus spore dust on roots at planting.

* Denotes significant differences (P = .05) between Pisolithus and Thelephora and SNS vs. SNS + spores on a pine species.

Table 2.--Survival and growth of Virginia pine seedlings after two years on a coal spoil (pH 3.8) in Rockcastle Co., Kentucky[1] tailored in a nursery with Pisolithus tinctorius (Pt) and Thelephora terrestris (Tt) ectomycorrhizae.

Ectomycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	$(Stem\ dia.)^2$ X height (volume)	Basidiocarp production Pt	Basidiocarp production Tt
Pisolithus	48.8	48.8	1.63	129.7	9	0
Thelephora	1.3[2]	19.0[2]	0.4[2]	3.0[2]	0	0

[1] Two experimental plots of each ectomycorrhizal treatment was destroyed by a logging deck. Data based on mean of four replicate plots (each with 40 seedlings) of each ectomycorrhizal treatment.

[2] Only two seedlings survived; all measurements are an average of these two seedlings and are not a treatment average.

Table 3.--Survival and growth of loblolly pine seedlings after two years on two coal spoils in Virginia[1] tailored in a nursery with *Pisolithus tinctorius* and *Thelephora terrestris* ectomycorrhizae

Ectomycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	(Stem dia)^2 X height (volume)
. . . . . Wise County, Virginia[2].				
Pisolithus	70	74	2.29	388.1
Thelephora	60	53	1.33	93.8
. . . . . Russell County, Virginia[3].				
Pisolithus	50	61	2.16	284.6
Thelephora	18	68	2.02	277.5

[1] On both sites many blocks of seedlings were either destroyed or damaged by flooding, cattle grazing, or trail bikes. These data from undisturbed blocks have no statistical basis due to inadequate replication.

[2] Spoil analyses were pH 3.3, 0.15% total N, 0.07% total S, and 3.8% organic matter, with 7, 50, 134, 55 and 35 lbs/acre of available P, K, Ca, Mg, and NO_3, respectively.

[3] Spoil analyses were pH 6.3, 0.07% total N, 0.02% total S, and 1.2% organic matter, with 139, 98, 1643, 528 and 36 lbs/acre of available P, K, Ca, Mg and NO_3, respectively.

all instances *Pisolithus* improved survival and growth. Examination of root systems revealed that *Pisolithus* totally dominated the roots on those seedlings originally ectomycorrhizal with it. Seedlings with *Thelephora* ectomycorrhizae at planting had nearly half of their ectomycorrhizae formed by *Pisolithus* after two years.

Owing to the destruction of the previous experiment, a planting was installed on another coal spoil in Virginia in 1974. Loblolly pine seedlings were tailored in the Athens nursery with *Pisolithus* or *Thelephora* ectomycorrhizae. The field design was a complete randomized plot with five plots (25 test trees each) of each ectomycorrhizal condition as used in Kentucky. We were fortunate to obtain second year data from this planting before vandals removed many trees. The results were again significant (Table 4). Although survival of seedlings was not significantly affected by *Pisolithus* ectomycorrhizae, growth as expressed by seedling volume (stem diameter^2 X height) was increased by over 150 percent. Both *Pisolithus* and *Thelephora* produced basidiocarps in their respective plots, but *Pisolithus* was the more prolific.

Table 4.--Survival and growth of loblolly pine seedlings after two
years on a coal spoil (pH 3.4) in Buchanan County, Virginia,
tailored in a nursery with Pisolithus tinctorius (Pt) and
Thelephora terrestris (Tt) ectomycorrhizae

Ectomycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	(Stem dia.)^2 X height (volume)	Basidiocarp production Pt	Tt
Pisolithus	90	101.4*	3.08*	961.9*	14	<1
Thelephora	79	81.2	2.16	378.9	<1	4*

*Denotes significant differences (P = .05) between mycorrhizal treatments.

Eroded Copperhill, Tennessee.--In 1974 our Work Unit entered into a
cooperative agreement with Cities Service Company to study the reforesta-
tion potential of severely eroded sections of the copper basin in southern
Tennessee. This basin has been devoid of acceptable vegetative cover
since the late 1800's. Our initial experiments (Berry and Marx, unpub-
lished data) involved planting Virginia and loblolly pines with Pisolithus
and Thelephora ectomycorrhizae from the Athens nursery on different sites.
Table 5 contains two-year data from one site. Survival of trees was good
and was not affected by ectomycorrhizal treatment. Pisolithus ectomycor-
rhizae significantly improved growth of both pine species. Incidence of
Pisolithus basidiocarps in the Pisolithus plots was high. Thelephora
basidiocarps occurred but were not recorded.

Table 5.--Survival and growth of Virginia and loblolly pine seedlings
after two years on an eroded site in Copper Basin, Tennessee,
tailored in a nursery with Pisolithus tinctorius and Thelephora
terrestris ectomycorrhizae

Pine species	Ectomycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	(Stem dia.)^2 X height (volume)	Pisolithus basidiocarps
Virginia	Pisolithus	95	52.3	1.46*	111.5*	24*
	Thelephora	100	44.8	1.15	59.3	2
Loblolly	Pisolithus	88	48.7	1.29*	81.0*	18*
	Thelephora	96	41.3	1.01	42.1	0

*Denotes significant differences (P =.05) between mycorrhizal treatments
of a pine species.

Clay spoils in Georgia.--In 1973, loblolly pine seedlings with Pisolithus and Thelephora ectomycorrhizae from our first study on soil infestation (Marx and Bryan 1975) were planted on a kaolin and a Fuller's earth strip-mined spoil. The seedlings were graded to similar height and stem diameters and planted in three blocks arranged in a randomized block design. Each block contained 20 seedlings of each ectomycorrhizal treatment and each seedling received 84 gm commercial 10-10-10 fertilizer broadcast yearly. Rust, caused by Cronartium fusiforme, severely damaged many seedlings. Data presented in Table 6 are average measurements taken after three years growth from the ten tallest, rust-free seedlings in each mycorrhizal treatment per block. Survival was good (90 to 95 percent) for all ectomycorrhizal treatments. Growth was significantly increased by Pisolithus ectomycorrhizae even though Pisolithus spread to the Thelephora seedlings after the first growing season. Most of this contamination was by Pisolithus actually growing vegetatively from roots across the eight-foot row spacing to the Thelephora seedlings. Pisolithus basidiocarps were present throughout all blocks after the second growing season. Regardless of initial ectomycorrhizal condition, after three years all seedlings had Pisolithus ectomycorrhizae (Table 6). However, there were more Pisolithus ectomycorrhizae on seedlings that were initially ectomycorrhizal with this symbiont at planting than on the Thelephora seedlings.

Table 6.--Growth of loblolly pine seedlings after three years on strip-mined clay spoils in Georgia tailored in the nursery with Pisolithus tinctorius and Thelephora terrestris ectomycorrhizae[1]

Ectomycorrhizal condition at planting	Height (cm)	Stem dia. (cm)	(Stem dia.)^2 X height (volume)	Percent seedlings with Pisolithus ectomycorrhizae
. . . . . Normal Kaolin Spoil				
Pisolithus	170*	5.0*	4250*	100
Thelephora	147	4.4	2846	100
. . . . . Fuller's Earth Spoil				
Pisolithus	159*	4.5	3220*	100
Thelephora	140	4.0	2240	100

[1] Measurements of the ten tallest, fusiform rust-free seedlings per replicate.

* Denotes significant differences (P = .05) between Pisolithus and Thelephora ectomycorrhizal treatments.

These results point out the potential benefits of tailoring pine
seedlings in the nursery with specific ectomycorrhizae for forestation
of various adverse sites. Many disturbed sites, including strip-mined
lands, are characterized by having soil factors which exert selective
pressures on symbiotic fungi. Fungi which can tolerate these factors
and are ecologically adapted to these sites should be used to "tailor"
seedlings prior to planting. Perhaps by using this silvicultural tech-
nique we can obtain seedlings with persistent and more physiologically
active root systems which will tolerate these adverse sites. Recently,
Muncie and coworkers (1975) detected elemental sulfur in Pisolithus
basidiocarps. Absorption and accumulation of sulfur from coal spoils
may in some fashion be related to the ecological adaptation of Pisoli-
thus to these high sulfur-containing sites.

An inherent difficulty in research of this nature has become
apparent--maintaining the integrity of the specific ectomycorrhizal
association on these sites. Pisolithus recolonizes roots of control
seedlings from airborne sources so rapidly that valid growth comparisons
between seedlings initially with and without Pisolithus at planting is
very erratic. Quite often many seedlings without Pisolithus at plant-
ing will have variable quantities of Pisolithus ectomycorrhizae by the
end of the first growing season in the field. Another problem en-
countered is that Pisolithus can spread very rapidly through soil and
contaminate nearby test seedlings. This necessitated use of different
plot designs to assure that seedlings with Pisolithus ectomycorrhizae
were isolated from control seedlings with sufficient distance (10 to 15
feet) to minimize this contamination.

Endomycorrhizal associations.--Research on plants with endomycor-
rhizae has been quite limited on strip-mined wastes. Daft and others
(in press) found abundant endomycorrhizae on roots of a variety of
herbaceous plants on anthracite and bituminous coal wastes in Pennsyl-
vania and bituminous wastes in Scotland. They identified and collected
spores of Gigaspora gigantea (= Endogone gigantea) from the Pennsylvania
wastes and found that they would infect and stimulate growth of corn
plants in spoil material. In addition to G. gigantea, other species of
Endogonaceae were also found on these spoils. They concluded that endo-
mycorrhizae may be essential for the survival and growth of herbaceous
plants on coal wastes.

Aldon (1975) found that endomycorrhizae significantly increased
survival and growth of four-wing saltbush (Altriplex canescens) on strip-
mined coal spoils in New Mexico. Size index (height X stem diameter) of
the mycorrhizal seedlings was 1493 and only 584 for the nonmycorrhizal
seedlings after the second year on the spoil site.

We have made observations on the incidence of endomycorrhizae on
herbaceous plants on strip-mined land. A variety of wild and cultured
grasses were endomycorrhizal to some degree on artificially and naturally
revegetated spoils in Kentucky and Virginia, as well as on clay spoils in

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Georgia. In the case of some grasses, not all seedlings had endomycorrhizae on the same site. Planted sycamore, sweetgum, maples, and black alder were heavily endomycorrhizal. The endomycorrhizae on these trees was probably on the roots before planting on the spoil; it is biologically significant, however, that the endomycorrhizae persisted.

Our first hardwood endomycorrhizae field study was planted in 1974 on kaolin spoils in Georgia (Bryan, unpublished data). Sycamore with and without endomycorrhizae formed by Glomus mosseae were grown in our experimental nursery in Athens. Seedlings were planted on two spoils in a randomized block design with 30 test seedlings per mycorrhizal condition in each of five blocks. Results after two years were striking (Table 7). Endomycorrhizae significantly improved survival on both sites and improved seedling growth by over 120 percent (seedling volume) on the Fuller's earth spoil. Glomus mosseae persisted very well on roots of the seedlings. However, after two years all sampled seedlings, even those initially nonmycorrhizal at planting, had many endomycorrhizae apparently formed by indigenous fungi.

Table 7.--Survival and growth of sycamore seedlings after two years on strip-mined clay spoils in Georgia with and without endomycorrhizae formed by Glomus mosseae in a nursery

Endomycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	$(Stem dia.)^2$ X height (volume)
. . . . .Normal Kaolin Spoil				
G. mosseae	49*	97.8	1.95	371.9
Nonmycorrhizal	26	104.1	2.09	454.7
. . . . . Fuller's Earth Spoil				
G. mosseae	62*	50.5*	0.97*	47.5*
Nonmycorrhizal	43	39.1	0.74	21.4

*
Denotes significant differences (P = .05) between mycorrhizal conditions.

Since some endomycorrhizal infection is apparent on wild grasses and volunteer trees on spoil material, the logical question to ask is what was the source of the initial inoculum? As mentioned above, endomycorrhizal fungi do not have highly effective means of dissemination. Most spread of these fungi is probably slow and caused mainly by moving water or soil, insects, birds, and, perhaps, animals (including man). Their presence on strip-mined lands may be accounted for through contamination of overburden material with the original topsoil. If some plants are dependent on, or at least stimulated by, endomycorrhizal infection, then perhaps by increasing the amount of available inoculum

revegetation of spoil material with these plants may be enhanced. Tailoring these plants in nurseries or in containers with specific endomycorrhizal fungi also should be thoroughly tested.

It appears that research on the value of endomycorrhizae to survival and growth of plants, including trees, on strip-mined lands should be emphasized on testing the variety of fungi which persist in the spoil. Perhaps an ecologically adapted endomycorrhizal fungus can be found which will have a similar potential to that of the ectomycorrhizal fungus Pisolithus tinctorius. Schenck and others (1975) and Schenck and Schroder (1974) have shown that some species of Glomus are more adapted to high soil temperatures than others. This suggests the possibility of selection among endomycorrhizal fungi for those species ecologically adapted to specific adverse sites having high soil temperatures. Techniques are currently being developed in Athens for large scale artificial soil infestation with specific endomycorrhizal fungi similar to those developed for ectomycorrhizal fungi.

It would be extremely interesting to determine the value of endomycorrhizae to pioneer plants. Are they pioneer plants because they do not require endomycorrhizae for normal growth? If sufficient quality and quantity of endomycorrhizal inoculum were present in spoil material would the same succession of herbaceous species prevail? There is tremendous potential for practical research on this aspect of endomycorrhizal associations.

CONCLUSIONS

It appears quite obvious that there is considerable merit to the concept of using mycorrhizal fungi to "tailor" seedlings prior to planting on disturbed lands. At this time Pisolithus tinctorius is the only ectomycorrhizal fungus with tolerance to soil adversity that has been used to "tailor" seedlings. However, when its broad host range and world-wide distribution is considered, it may be the best fungal symbiont for this purpose. After an extensive literature search, contacts with world herbaria, and controlled experimentation, we found the host range for P. tinctorius to include four species of Betula, twelve species of Eucalyptus, nine species of Quercus, 37 species of Pinus, and Douglas-fir. Its distribution is world-wide and has been confirmed as naturally occurring in most of the fifty states in the United States. Pisolithus is readily culturable on simple growth media under laboratory conditions and can withstand the rigors of inoculum leaching and mechanical incorporation into soil. Many other species of ectomycorrhizal fungi do not have these traits and, therefore, may be more difficult to use successfully in soil infestation.

There is a need for extensive research on the use of specific species of endomycorrhizal fungi on roots of grasses, shrubs and hardwood tree species in reclamation of disturbed lands. Although research has just begun on hardwood endomycorrhizae, their potential in forestation of disturbed lands may be greater than that of Pisolithus ectomycorrhizae on pines.

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THE ROLE OF MYCORRHIZAE IN FOREST PRODUCTION

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Abstract—Use of specific, highly beneficial mycorrhizal fungi has great potential benefits in tree nurseries and in artificial regeneration programs. *Pisolithus tinctorius*, an ectomycorrhizal fungus of many conifers, oak and eucalyptus, shows tremendous promise for improving survival and growth of trees on a variety of adverse (coal spoils, eroded sites, etc.) and routine reforestation sites. Commercial inoculum of this fungus may soon be available for artificially infesting nursery soils to tailor roots of tree seedlings. Current research also indicates that endomycorrhizae are essential to growth of most hardwoods. Research will continue on endomycorrhizae with the objective of improving nursery quality of hardwood seedlings and their performance in the field.

Microorganisms are present in great numbers near the feeder roots of trees, and they play vital roles in numerous physiological processes. These dynamic microbial processes include saprophytism, pathogenicity and symbiosis. The most widespread symbiosis of trees is the mycorrhizal association between root-inhabiting fungi and the feeder roots of trees. Mycorrhizal associations are so common under natural soil conditions that a nonmycorrhizal tree is the exception.

TYPES OF MYCORRHIZAE ON FOREST TREES

ECTOMYCORRHIZAE

This type occurs naturally on many important forest tree species around the world. All members of the gymnosperm family *Pinaceae* (pine, spruce, fir, larch, hemlock, etc.) as well as certain angiosperms (willow, poplar, aspen, hickory, pecan, oak, birch, beech, eucalypt, etc.) normally form ectomycorrhizae. Some of these trees can be either ectomycorrhizal or endomycorrhizal, depending on soil conditions. Ectomycorrhizal infection is initiated from spores or hyphae (propagules) of the fungal symbionts inhabiting the rhizosphere of the feeder roots. Propagules are stimulated by root exudates and grow over the feeder root surface and form a fungus mantle. Following mantle development, hyphae develop around root cortical cells and form the Hartig net. These hyphae may completely replace the middle lamellae between the cortical cells. The Hartig net is the main distinguishing feature of ectomycorrhizae. Ectomycorrhizal roots may be unforked, bifurcate, multiforked (coralloid), nodular, or in other shapes. The color of an ectomycorrhiza is determined by the color of the hyphae of the fungal symbiont and may be brown, black, white, red, yellow, or blends of these colors. Individual hypha, strands of hyphae, or rhizomorphs may radiate from the fungus mantles into the soil and to the base of the fruit bodies of the fungi.

Most fungi which form ectomycorrhizae with forest trees are Basidiomycetes that produce mushrooms or puffballs (fruit bodies). Certain Ascomycetes such as truffles also form mycorrhizae, however. Over 2100 species of these fungi can form ectomycorrhizae on trees in North America. The fruit bodies of these fungi produce billions of spores that are widely disseminated by wind and water. Most ectomycorrhizal fungi are dependent on their hosts for simple carbohydrates, amino acids, vitamins, etc., necessary to complete their life cycles. Ectomycorrhizal development, therefore, is a prerequisite for fruit body production by these fungi; not all fungi which form mushrooms and puffballs, however,

are ectomycorrhizal. Many of these fungi are saprophytes that play important roles in the decomposition of organic matter and the mineral cycle in forest soils. Certain other species are pathogenic to trees.

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest, a single tree species, an individual tree, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza. Even as a single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots, a single fungus can enter into ectomycorrhizal association with numerous tree species. Some fungi are apparently host-specific; others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families.

Ectomycorrhizal fungi aid the growth and development of trees. For some trees, such as *Pinus*, they are indispensable for growth under natural conditions. Trees with abundant ectomycorrhizae have a much larger, physiologically active, root-fungus area for nutrient and water absorption than trees with few or no ectomycorrhizae. This increase in surface area comes both from the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae of the fungus symbionts from the ectomycorrhizae into the soil. These extramatrical hyphae function as additional nutrient and water-absorbing entities and assure maximum nutrient capture from the soil by the host. Ectomycorrhizae are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium in the fungus mantles more rapidly, and for longer periods of time, than nonmycorrhizal feeder roots. Ectomycorrhizal fungi help break down certain complex minerals and organic substances in the soil and transmit nutrients from these materials to the tree. Ectomycorrhizae also appear to increase the tolerance of trees to drought, high soil temperatures, soil toxins (organic and inorganic), and extremes of soil pH caused by high levels of sulfur or aluminum. Ectomycorrhizae deter infection of feeder roots by root pathogens, such as species of *Pythium* or *Phytophthora*. Hormone relationships induced by fungal symbionts cause ectomycorrhizal roots to have greater longevity (length of physiological activity) than nonmycorrhizal roots.

ENDOMYCORRHIZAE

This type is formed on most economically important agronomic and forage crops, as well as fruit and nut trees. Important forest trees such as maple, elm, gum,

ash, sweetgum, sycamore, and black walnut form endomy-
corrhizae. Some trees, such as alder, can form both
endo- and ectomycorrhizae. Endomycorrhizal fungi form
a loose network of hyphae on feeder root surfaces and
do not develop the dense fungus mantle of ectomycor-
rhizae. These fungi often form large, conspicuous,
thick-walled spores both on the root surfaces and in
the rhizosphere, and sometimes in feeder root tissues.
Hyphae of endomycorrhizal fungi penetrate the cell walls
and progress into the cortical cells of the root.
These infective hyphae may develop specialized absorb-
ing or nutrient-exchanging structures (haustoria) called
arbuscules in the cytoplasmic matrix of the cortical
cells. Thin-walled, spherical-to-ovate vesicles may
also be produced in cortical cells by these fungi. The
term "vesicular-arbuscular" mycorrhizae has been coined
to denote this type of endomycorrhizae. As in ectomy-
corrhizae, endomycorrhizal fungus infection rarely
occurs in meristematic or vascular tissues. Endomycor-
rhizal infection, however, does not cause major morpho-
logical changes in roots. Endomycorrhizae, therefore,
cannot be detected with the unaided eye.

The fungi which form endomycorrhizae with trees are
mainly Phycomycetes. They do not produce large, above-
ground fruit bodies or wind-disseminated spores as do
most ectomycorrhizal fungi, but some of them produce
large spores on or in roots. Some species also produce
large sporocarps (5-10 nm diameter) containing many
spores on roots. These fungi spread through the soil by
growing from feeder root to feeder root; they are also
disseminated by moving water, soil, insects, or animals.
Many endomycorrhizal fungi of trees belong to the family
Endogonaceae. Several genera and species have been
identified; many more undoubtedly exist. They are so
widespread that it is nearly impossible to find natural
soils anywhere in the world that do not contain them.
Spores of these fungi are able to survive in soil for
years without a host. Based on the limited research
done on endomycorrhizal fungi, most species appear to
have very broad host ranges. For example, *Glomus
mosseae* is known to form endomycorrhizae with cotton,
corn, pepper, soybean, and sorghum, as well as sycamore,
sweetgum, citrus, peach, black locust, green ash, black
cherry, boxelder, sugar maple, and red maple. It un-
doubtedly can infect numerous other plant species.

Only limited research has been conducted to determine
the value of endomycorrhizae to forest trees. Avail-
able research results indicate that endomycorrhizal
roots absorb nutrients better than nonmycorrhizal roots.
This applies especially to the absorption and utiliza-
tion of phosphorus. The extensive network of hyphae of
these fungi growing from endomycorrhizae captures
nutrients from large volumes of soil that are rarely
explored by nonmycorrhizal roots. Recently, it was
found that certain hardwood trees depend on endomycor-
rhizae for normal growth like *Pinus* species depend on
ectomycorrhizae. Recent research at our Athens Labora-
tory indicates that sweetgum and perhaps black cherry,
boxelder, green ash, and red maple require endomycor-
rhizae for establishment and normal growth in nurseries.

ECTENDOMYCORRHIZAE

This type of mycorrhizae has the features of both ecto-
and endomycorrhizae. Ectendomycorrhizae have a limited
distribution in forest soils and are found primarily on
roots of normally ectomycorrhizal trees. Very little
is known about the species of fungi involved or their
importance to tree growth because little research has
been done on them.

FACTORS AFFECTING MYCORRHIZAL DEVELOPMENT

Many factors affect mycorrhizal development. It is
necessary, however, to separate those which affect the
tree from those which affect the fungal symbionts.
Generally, any soil or above-ground condition which
influences root growth also influences mycorrhizal
development. A susceptible feeder root must be formed
by the tree before mycorrhizal infection can occur.
The main factors influencing susceptibility of tree
roots to mycorrhizal infection appear to be photosyn-
thetic potential and soil fertility. High light in-
tensity and low to moderate soil fertility enhance
mycorrhizal development; the other extremes of these
conditions (light intensity below 20 percent of full
sunlight and excessively high soil fertility) reduce,
or may even prevent, mycorrhizal development. Light
intensity and fertility appear to influence either the
biochemical status of feeder roots, such as controlling
levels of simple sugars, or the synthesis of new feeder
roots, both of which are prerequisites to symbiotic
infection. Roots growing rapidly because of high soil
fertility contain few simple sugars and they are not
highly susceptible to infection.

The factors which affect the fungal symbionts directly
are those which regulate survival of the fungus in the
soil or its growth on roots. Extremes of soil tempera-
tures, pH, moisture, etc., and presence of antagonistic
soil microorganisms can affect the survival of sym-
bionts and thereby influence the mycorrhizal potential
of the soil. Mycorrhizal fungi cannot grow and re-
produce in soil unless they are in symbiotic associa-
tion with plant roots. These fungi are capable, how-
ever, of surviving in a dormant condition for several
years without a plant host.

Several excellent texts have been published which dis-
cuss these different aspects of mycorrhizae. [1,2,3,4]

MYCORRHIZAE AND AFFORESTATION PRACTICES

Over 3000 papers have been published on mycorrhizae,
and most of these relate to forest trees. [5] This
research has shown the complexity of mycorrhizae in
forest soil ecosystems. Many forest trees, such as
Pinus, cannot grow without ectomycorrhizae. Recent
findings imply that any kind of ectomycorrhizae is
better than no ectomycorrhizae at all, especially on
pines. This point has practical significance to
afforestation programs with normally ectomycorrhizal
trees in areas of the world where their symbiotic fun-
gi do not occur naturally. Mikola[6] discussed the need
for a parallel introduction of the essential ectomycor-
rhizal fungi with these trees, particularly *Pinus*, if
afforestation is to succeed. In many areas of the
world, ectomycorrhizal trees and their symbiotic fungi
do not occur naturally. Such areas include the high
Andes of Peru,[7] regions of Australia[8] and Asia,[9]
subalpine areas of Austria,[10] Puerto Rico,[11] Africa,[12]
former agricultural soils of Poland,[13] oak shelterbelts
on the steppes of Russia,[14] and former treeless areas
of the United States.[15] Forestation attempts there
were either total or near failures until ectomycorrhizae
occurred on tree roots. Symbiotic root infection was
insured either by introducing soil containing ectomy-
corrhizal fungi or by manipulating soil containing low
levels of indigenous symbiotic fungi to encourage their
buildup on roots.

Natural soils of the world which have supported vege-
tation in the past have not been reported void of endo-
mycorrhizal fungi. The broad host range of most species
of endomycorrhizal fungi normally ensures endomycor-
rhizal development on desirable plants. Failure in

establishment of normally endomycorrhizal trees because of a deficiency of endomycorrhizal infection in normal soils has not been reported. However, slow early growth of certain hardwood species could be the result of inadequate amounts of endomycorrhizae at planting time.

ECTOMYCORRHIZAE AND REVEGETATION OF ADVERSE SITES

With the exception of a few reports, very little is known about the ectomycorrhizal fungi on trees growing on strip-mine spoils, borrow pits, severely eroded lands, and other disturbed sites. Several questions about forestation of such areas must be answered. Can indigenous and recolonizing ectomycorrhizal fungi survive the soil conditions of these sites? Is there significant potential for ecological selection among the vast numbers of species of ectomycorrhizal fungi to assure that adaptable ones will maintain themselves on the roots of trees in these disturbed sites? Limited research has been done on ectomycorrhizal associations of trees on strip-mine lands and mining wastes.

In 1966, Schramm[16] reported on plant colonization of anthracite wastes in Pennsylvania. He concluded that early ectomycorrhizal development was essential on this waste material for establishment of *Betula lenta, B. populifolia, Pinus rigida, P. virginiana, Populus tremuloides, Quercus rubra,* and *Q. velutina* seedlings. The only generally successful original plant colonists of this bare and predominantly nitrogen-deficient waste were nitrogen-fixing plants and certain ectomycorrhizal tree species. Schramm suggested from strong evidence that due to their year-round effectiveness, evergreen (pines) trees should receive special attention. Seeded and volunteer seedlings of these tree species became chlorotic and soon died when they did not have ectomycorrhizae. The majority of surviving seedlings, especially those growing well, were heavily ectomycorrhizal. The main Basidiomycetes observed by Schramm associated with seedlings of these tree species were *Inocybe lacera, Thelephora terrestris, Pisolithus tinctorius, Amanita rubescens,* and *Scleroderma aurantium,* all of which form ectomycorrhizae on various trees.[17] Schramm[16] traced the extensively developed mycelial strands of *P. tinctorius* from ectomycorrhizae through large volumes of anthracite wastes to the base of the fungus basidiocarp. These large, brilliant gold-yellow mycelial strands were easily traced with the naked eye through the contrasting dark wastes. Some strands were traced through waste material as far as 15 feet from the seedlings to the basidiocarp. The ectomycorrhizae he associated with *Pisolithus* were also gold-yellow in color and prolifically branched. Seedlings with *P. tinctorius* ectomycorrhizae were the most vigorously growing seedlings and, in most cases, these ectomycorrhizae were the first detected on seedling roots. The other species of ectomycorrhizal fungi appeared on roots and produced basidiocarps primarily after litter had accumulated under the older seedlings.

Schramm's observations strongly suggest that a few ectomycorrhizal fungi may be capable of ecologically adapting to conditions on the anthracite wastes. High soil temperatures found on coal wastes and spoils could be a limiting factor in the establishment of specific mycorrhizal fungi. Marx and coworkers[18] found that *P. tinctorius* formed more ectomycorrhizae on *P. taeda* seedlings at a constant soil temperature of 34° C than at 14, 19, 24, or 29° C. In this study, *Thelephora terrestris* did not form ectomycorrhizae at soil temperatures of 34° C and formed more between 14 and 24° C than at 29° C. *T. terrestris,* one of the major ectomycorrhizal fungi on pine seedlings in nurseries through-

out the United States, is also found on pines planted on coal spoils. It was on seedling roots from the nursery prior to planting on the spoil. In a later study, Marx and Bryan[19] found that *P. taeda* seedlings with *Pisolithus* ectomycorrhizae survived and grew as well at 40° C as they did at 24° C. Seedlings with *Thelephora* ectomycorrhizae or without ectomycorrhizae survived poorly and did not grow at 40° C. This temperature tolerance may explain why *Pisolithus* is one of the primary symbionts on young volunteer seedlings growing on anthracite wastes. Schramm[16] recorded soil temperatures between 35° C and 65° C in wastes at a depth of 6 to 7 cm. Perhaps *Pisolithus* was dominant because high soil temperatures restricted earlier establishment of the other symbiotic fungi.

We have extensively examined various strip-mine spoils in the East. *Pisolithus* basidiocarps and its characteristic gold-yellow ectomycorrhizae and mycelial strands were found to be the predominant, if not the only, ectomycorrhizal fungus on roots of *Pinus taeda, P. virginiana, P. resinosa,* and several species of *Betula* and *Quercus* on coal spoils in Indiana, Ohio, Pennslyvania, West Virginia, Virginia, Kentucky, Tennessee, and Alabama; *P. echinata* and *P. taeda* on strip-mine kaolin spoils in Georgia; and *P. rigida* on iron wastes in Virginia. Some of these spoils had a soil reaction as low as pH 2.4, although most had a reaction between pH 3.5 and 5.5. Basidiocarps of *Pisolithus* have also been reported on coal spoils under *B. lenta, B. pendula, B. populifolia, Populus grandidenta, P. tremuloides,* and *Salix humilis* in West Germany,[20] *Pinus banksiana* in Missouri,[21] and *Pinus* spp. in Indiana and Tennessee.[22] We have also found basidiocarps of *Pisolithus* around pines growing in the copper basin of Tennessee and on borrow pits throughout the Southeastern United States.

Following is an example of the prevalence of *Pisolithus* on disturbed lands. On a kaolin spoil in central Georgia, basidiospores of *Pisolithus* were collected from basidiocarps under 5-year-old loblolly pines. In less than 12 man-hours, over 12 kilograms of spores were extracted from basidiocarps. There are approximately 1.1 billion spores per gram; this one collection contained approximately 12.5 trillion basidiospores. Basidiospores can be used as inoculum for ectomycorrhizal development on pines.[23, 24]

NURSERY MANIPULATION OF ECTOMYCORRHIZAL FUNGI

Based on Schramm's observations and these other reports, it seems that *Pisolithus* may be more beneficial to the establishment of ectomycorrhizal trees on strip-mined and other disturbed lands than other species of ectomycorrhizal fungi. Making the assumption that *Pisolithus* ectomycorrhizae are instrumental in tree establishment and maintenance on strip-mine spoils, we began to develop techniques to tailor seedlings in the nursery with *Pisolithus* ectomycorrhizae. The working premises were simple: Why wait for natural inoculum to establish *Pisolithus* ectomycorrhizae on tree seedlings on these adverse sites? Tree survival and growth can be improved by having *Pisolithus* ectomycorrhizae on the seedlings from the nursery prior to planting on disturbed lands.

Recently, the tree-tailoring techniques were perfected and demonstrated on several pine species in conventional tree nurseries.[24] Briefly, the techniques involve the production of either a pure culture of vegetative mycelial inoculum of *P. tinctorius* in a mixture of vermiculite, peat moss and nutrient substrate or basidiospores mixed with a physical carrier, such as moist vermiculite. The inocula are used to infest fumigated

nursery soil. Effective soil fumigation (methyl bromide) shortly before soil infestation and the maintenance of reasonable levels of soil fertility through the growing season appear to be the only prerequisites for successfully tailoring pine seedlings with *Pisolithus* ectomycorrhizae.

The introduction of *Pisolithus* into nursery soils has significantly improved pine seedling quality in comparison to routine seedlings with naturally occurring *Thelephora* ectomycorrhizae. Growth increases in the nursery between 100 and 150 percent after one growing season have been demonstrated with seedlings of *P. taeda*, *P. strobus*, and *P. virginiana* in North Carolina following soil infestation and ectomycorrhizal development by *Pisolithus*.[24]

In Oklahoma, an earlier deficiency of ectomycorrhizal fungi was corrected by introducing pure cultures of *Pisolithus* and *Thelephora* inocula (unpublished data). Soil infestation with the fungi increased the number of plantable-sized seedlings of *P. taeda* by 145 percent and the size (fresh weights) of the plantable stock by another 140 percent. Research was recently completed in two nurseries in Virginia (unpublished data) that had an abundance of naturally occurring fungi. Artificial soil infestation with *Pisolithus* and *Thelephora* increased seedlings size of *P. taeda* by 57 and 31 percent in one nursery and 40 and 20 percent in the other nursery, respectively. After a few more years research, we are confident that ectomycorrhizal deficiencies in large nurseries can be corrected by using pure cultures of highly beneficial symbionts. In the past, these ectomycorrhizal deficiencies were corrected by adding forest litter and humus to the nursery soil. This practice sometimes failed to produce adequate mycorrhizal infection and, in some instances, inadvertently introduced a considerable number of pests, such as weeds and disease-causing organisms into nurseries.

We are currently working with Abbott Laboratories, Chicago, to ascertain the feasibility of producing vegetative mycelial inoculum of *P. tinctorius* in large capacity fermentors for commercial use. Within the next year, this inoculum will be tested by us to determine its viability for ectomycorrhizal synthesis. The production of this inoculum in large quantities should be relatively inexpensive--the added cost should be readily offset by improved seedling quality in the nursery. When available, this inoculum should assist in seedling production in nurseries of a vast number of tree species throughout the world. Tests have confirmed that *P. tinctorius* forms ectomycorrhizae with *Abies procera*, *Betula pendula*, *Carya illinoensis*, 11 species of *Eucalyptus*, 30 species of *Pinus*, *Pseudotsuga menziesii* var. *menziesii*, and 2 species of *Quercus*. It has been reported fruiting under 3 additional species of *Betula*, 2 species of *Eucalyptus*, 8 species of *Pinus*, 8 species of *Quercus*, *Populus tremuloides*, *Pseudotsuga grandidenta*, and *Salix humilis*.[26] Not one ectomycorrhizal tree species tested by this author failed to form ectomycorrhizae with *P. tinctorius* under controlled conditions. The natural occurrence of *P. tinctorius* has been confirmed in 33 countries of the world and in 38 states of the United States. It is found with trees growing in urban areas, orchards, and forests, as well as on adverse sites. It occurs rarely in nurseries, particularly in those practicing soil fumigation.

The practicability of introducing *Pisolithus* and other ectomycorrhizal fungi into the nearly sterile root substrate of containerized seedlings is also being examined. Preliminary information suggests that growth in containers and field performance of these seedlings can be improved with specific ectomycorrhizae.[27,28] Since container stock is soon expected to account for as much as 20 percent of the seedlings used in reforestation in North America,[29] use of specific mycorrhizal fungi with containerized tree stock could be very significant.

PERFORMANCE OF SEEDLINGS WITH SPECIFIC ECTOMYCORRHIZAE ON ADVERSE SITES

Since techniques to tailor tree seedlings with *Pisolithus* and other ectomycorrhizae are new, only limited data on seedling performance on disturbed lands are available. Since the winter of 1973, our group in Athens and our cooperators have outplanted nearly 30,000 tree seedlings with specific ectomycorrhizae on a variety of disturbed surface areas. Following are some of the results.

SEEDLING PERFORMANCE ON COAL SPOILS

One of our first tests was installed in Kentucky in 1973 on a very toxic (pH 3.8) coal spoil that had been unsuccessfully planted with pine several times. Virginia pine seedlings were grown with either *Pisolithus* or *Thelephora* ectomycorrhizae in our experimental nursery in Athens.[25] The results after two years were dramatic (Table 1). Only two of the 160 seedlings with *Thelephora* ectomycorrhizae survived, whereas 78 of the 160 seedlings with *Pisolithus* ectomycorrhizae survived. More significant is the fact that *Pisolithus* seedlings grew extremely well while the remaining *Thelephora* seedlings were no larger after two years on site than they were at planting. Basidiocarp production by *Pisolithus* in the *Pisolithus* plots was prolific.

TABLE I

Survival and growth of Virginia pine seedlings after two years on a coal spoil in Kentucky. Seedlings were tailored in a nursery with *Pisolithus tinctorius* or *Thelephora terrestris* ectomycorrhizae.

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	Seedling volume (cm³)[1]
Pisolithus	49*	49*	1.6*	130*
Thelephora	1	19	0.4	3

[1] Seedling volume (cm³) = (stem diameter)² X height.

* Denotes significant differences (P = .05) between means for mycorrhizal treatments.

Another planting was installed on a toxic (pH 3.4) coal spoil in Virginia in 1974. Loblolly pine seedlings were tailored in the Athens nursery with *Pisolithus* or *Thelephora* ectomycorrhizae. We were fortunate to obtain second year data from this planting before vandals removed many trees. The results were again highly significant (Table 2). Although survival of seedlings was not affected by *Pisolithus* ectomycorrhizae, growth as expressed by seedling volume was increased by over 150 percent. Both *Pisolithus* and *Thelephora* produced basidiocarps in their respective plots, but *Pisolithus* was more prolific.

The last example is another toxic (pH 3.9) coal spoil in Kentucky. This spoil was unsuccessfully planted twice using standard nursery grown loblolly pine seedlings. Loblolly and shortleaf pine seedlings were tailored with *Pisolithus* and *Thelephora* ectomycorrhizae in our Athens nursery and graded to similar sizes. In this test, we measured height and stem diameters shortly

after planting and after the first and second growing seasons. The data were transformed into plot volume indices (PVI) with the formula PVI = (stem diameter)^2 X height X number of surviving trees per replicate plot. We feel this index is excellent for comparing total growth response because it integrates survival, height and stem diameter into a single value. Figure 1 shows the growth of these seedlings through two years. There was no difference in the PVI at planting due to initial ectomycorrhizal condition. After the first growing season, plots of loblolly pine with *Pisolithus* ectomycorrhizae had 2.5 times more volume than plots with *Thelephora* ectomycorrhizae; plot volumes of shortleaf pine were double with *Pisolithus* ectomycorrhizae. Differences after two years were greater. Plot volumes for loblolly pine with *Pisolithus* ectomycorrhizae were 4.8 times greater than for loblolly pine with *Thelephora* ectomycorrhizae. *Pisolithus* ectomycorrhizae more than tripled PVI of shortleaf pine. During the second growing season on this coal spoil, *Pisolithus* produced over 30 fruit bodies per loblolly pine plot and 18 fruit bodies per shortleaf pine plot. Only three fruit bodies of *Thelephora* were found in all the *Thelephora* plots. These results indicate better survival potential of *Pisolithus* on this adverse site.

TABLE II

Survival and growth of loblolly pine seedlings after two years on a coal spoil in Virginia. Seedlings were tailored in a nursery with *Pisolithus tinctorius* or *Thelephora terrestris* ectomycorrhizae.

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	Seedling volume (cm^3) [1]
Pisolithus	90	101.4*	3.08*	961.9*
Thelephora	79	81.2	2.16	378.9

[1] Seedling volume (cm^3) = (stem diameter)^2 X height.

* Denotes significant differences (P= .05) between means for mycorrhizal treatments.
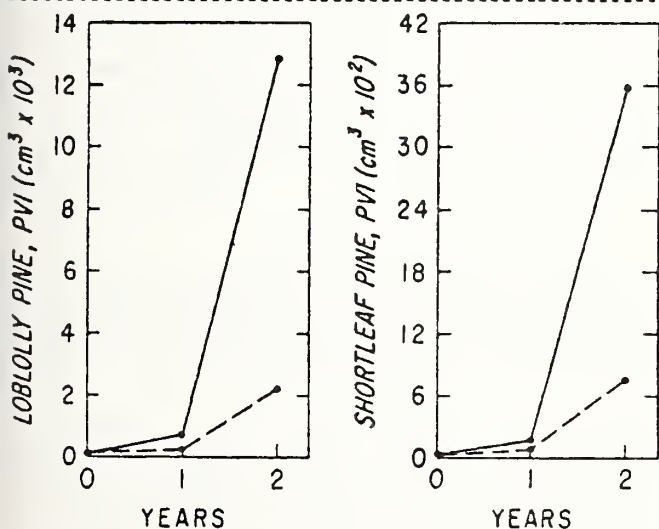
FIGURE 1. Plot volume indices (PVI) of loblolly and shortleaf pine seedlings after two years on a coal spoil in Kentucky. Seedlings were tailored with *Pisolithus* (•——•) or *Thelephora* (•--•) ectomycorrhizae at planting. Each point represents the mean of 25 test trees initially planted in each of 5 plots [PVI (cm^3) = (stem diameter)^2 X height X number surviving trees per plot].

SEEDLING PERFORMANCE ON ERODED SITES IN COPPERHILL, TENNESSEE

In 1974, we entered into a cooperative agreement with Cities Services Company to study the reforestation potential of severely eroded sections of the Copper Basin in southern Tennessee. This Basin has been devoid of acceptable vegetation since the late 1800's. Our initial experiments (unpublished data) involved Virginia and loblolly pines with *Pisolithus* or *Thelephora* ectomycorrhizae. Table 3 contains two-year data from one typical site. Survival of trees was good and was not significantly affected by ectomycorrhizal treatment. However, after two years, *Pisolithus* ectomycorrhizae significantly improved growth of both pine species. Incidence of *Pisolithus* basidiocarps in the *Pisolithus* plots was high. *Thelephora* basidiocarps occurred rarely.

TABLE III

Survival and growth of Virginia and loblolly pine seedlings after two years on an eroded site in Copperhill, Tennessee. Seedlings were tailored in a nursery with *Pisolithus tinctorius* or *Thelephora terrestris* ectomycorrhizae.

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	Seedling volume (cm^3) [1]
	. . Virginia Pine . .			
Pisolithus	95	52.3	1.46*	111.5*
Thelephora	100	44.8	1.15	59.3
	. . Loblolly Pine . .			
Pisolithus	88	48.7	1.29*	81.0*
Thelephora	96	41.3	1.01	42.1

[1] Seedling volume (cm^3) = (stem diameter)^2 X height.

* Denotes significant differences (P = .05) between means for mycorrhizal treatments of a pine species.

SEEDLING PERFORMANCE ON CLAY SPOILS

In 1973, loblolly pine seedlings with *Pisolithus* or *Thelephora* ectomycorrhizae were planted on spoils from kaolin and Fuller's earth strip-mines near Macon, Georgia. Each seedling received 84 gm commercial 10-10-10 fertilizer broadcast yearly. Rust, caused by *Cronartium fusiforme*, severely damaged many seedlings. Data presented in Table 4 are average measurements taken after three years of growth from the 10 tallest, rust-free seedlings in each mycorrhizal treatment per block. Survival was good (90 to 94 percent) for all treatments. Growth was significantly increased by *Pisolithus* ectomycorrhizae even though *Pisolithus* spread to the *Thelephora* seedlings after the first growing season. Most of this contamination was by *Pisolithus* growing vegetatively from roots across the eight-foot row spacing to the *Thelephora* seedlings. *Pisolithus* basidiocarps were present throughout all blocks after the second growing season. Regardless of initial ectomycorrhizal condition, all seedlings had *Pisolithus* ectomycorrhizae after three years. However, there were more *Pisolithus* ectomycorrhizae on seedlings that were ectomycorrhizal with this symbiont at planting than on the *Thelephora* seedlings.

TABLE IV

Growth of loblolly pine seedlings after three years on strip-mine clay spoils in Georgia. Seedlings were tailored in the nursery with *Pisolithus tinctorius* or *Thelephora terrestris* ectomycorrhizae.

Mycorrhizal condition at planting	Height (cm)	Stem dia. (cm)	Seedling volume (cm³)[1]
. . Normal Kaolin Spoil . .			
Pisolithus	170*	5.0*	4250*
Thelephora	147	4.4	2846
. . Fuller's Earth Spoil . .			
Pisolithus	159*	4.5	3220*
Thelephora	140	4.0	2240

[1] Seedling volume (cm^3) = $(stem\ diameter)^2$ X height.

* Denotes significant differences (P = .05) between means of *Pisolithus* and *Thelephora* ectomycorrhizal treatments within each site.

In 1975, another outplanting was installed on kaolin spoils (unpublished data). This site was covered with 7.5 cm of forest soil before the loblolly pine seedlings were planted. The seedlings had ectomycorrhizae formed by *Pisolithus*, *Thelephora* or *Cenococcum graniforme*. After planting, half the seedlings were fertilized with 170 gm of 10-10-10 fertilizer applied in slits on two sides of each seedling. The remaining seedlings were not fertilized. *Cenococcum* was tested because it has been reported to increase drought tolerance of trees;[30,31] kaolin spoils are droughty. Table 5 shows survival and growth increments of these seedlings after one year. *Pisolithus* ectomycorrhizae increased survival and also improved growth of loblolly pines on this spoil. Seedlings with *Cenococcum* ectomycorrhizae generally grew better than seedlings with *Thelephora* ectomycorrhizae. Fertilizer decreased survival overall, but improved growth of seedlings with *Cenococcum* and *Thelephora* ectomycorrhizae. Seedlings with *Pisolithus* ectomycorrhizae outgrew other seedlings. They also grew better without than with fertilizer.

TABLE V

First year growth increment of loblolly pine seedlings with *Pisolithus tinctorius*, *Cenococcum graniforme* or *Thelephora terrestris* ectomycorrhizae at planting, with or without fertilizer, on a kaolin spoil covered with 7.5 cm of forest soil in Georgia.[1]

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	PVI (cm³)[2]
. . Fertilized (170 gm/seedling 10-10-10) . .				
Pisolithus	73b	6.6a	0.23ab	6.1b
Cenococcum	55b	7.4a	0.21abc	4.3b
Thelephora	65b	4.1b	0.18abc	2.1b
. . Nonfertilized . .				
Pisolithus	94a	7.0a	0.27a	11.5a
Cenococcum	83a	5.6ab	0.16bc	2.9b
Thelephora	73b	3.4b	0.12b	0.9b

[1] Means sharing the same letter are not significantly different at P = 0.05.

[2] Plot volume index (PVI) = $(stem\ diameter)^2$ X height X number of surviving trees (30 trees/replicate).

Tailoring pine seedlings in the nursery with specific ectomycorrhizae has obvious potential benefits for forestation of various adverse sites. Many disturbed sites, including strip-mined lands, have soil factors that exert selective pressures on symbiotic fungi. Fungi that can tolerate these factors and are ecologically adapted to these sites should be used to tailor seedlings prior to planting. Perhaps by using this technique we can obtain seedlings with more physiologically active root systems which can tolerate these adverse sites. Recently, Muncie and coworkers[32] detected elemental sulfur in *Pisolithus* basidiocarps. Absorption and accumulation of sulfur from coal spoils may be related to the ecological adaptation of *Pisolithus* to these sites with high sulfur contents.

In this field of research, it has proved difficult to maintain the integrity of the specific ectomycorrhizal associations. *Pisolithus* recolonizes roots of control seedlings from airborne sources so rapidly that valid growth comparisons between seedlings initially with and without *Pisolithus* at planting are often difficult. Frequently many seedlings without *Pisolithus* at planting will have variable quantities of *Pisolithus* ectomycorrhizae by the end of the first growing season in the field. *Pisolithus* can spread vegetatively very rapidly through soil and contaminate nearby test seedlings. Plots of seedlings with *Pisolithus* ectomycorrhizae must be isolated from control seedlings by 10 to 15 feet to minimize this contamination.

ENDOMYCORRHIZAE AND REVEGETATION OF ADVERSE SITES

Research on endomycorrhizae of trees on adverse sites has been quite limited. Daft and others[33] found abundant endomycorrhizae on roots of a variety of herbaceous plants on anthracite and bituminous coal spoils in Pennsylvania and bituminous spoils in Scotland. They identified and collected spores of *Gigaspora gigantea* from the Pennsylvania spoils and found that they would infect and stimulate growth of corn. In addition to *G. gigantea*, several other species of *Endogonaceae* were also found on these spoils. They also found that *Fragaria vesca*, a native plant on the spoil in Scotland, responded to high levels of endomycorrhizae produced by indigenous symbionts. It was concluded that endomycorrhizae were essential for the survival and growth of herbaceous plants on these coal spoils.

Aldon[34] found that endomycorrhizae significantly increased survival and growth of four-wing saltbush (*Altriplex canescens*) on strip-mined coal spoils in New Mexico. Endomycorrhizal seedlings were 1.5 times larger than the nonmycorrhizal seedlings after the second year.

We have observed the incidence of endomycorrhizae on herbaceous plants on adverse sites in the Eastern United States. A variety of wild and cultured grasses were endomycorrhizal to some degree on artificially and naturally revegetated coal spoils in Kentucky and Virginia, as well as on kaolin and Fuller's earth spoils in Georgia. In the case of some grasses, not all seedlings on the same site had endomycorrhizae. Planted sycamore, sweetgum, maple, and black alder were heavily endomycorrhizal. The endomycorrhizae on these trees were probably on the roots before planting on the spoil; it is biologically significant, however, that the endomycorrhizal fungi persisted.

NURSERY MANIPULATION
OF ENDOMYCORRHIZAL FUNGI

Quality seedlings of hardwoods which normally form endomycorrhizae are difficult to grow in nurseries following soil fumigation (methyl bromide). Quite frequently the seedlings, especially sweetgum, must be grown for two years before they reach sufficient size for outplanting. Effective soil fumigation can eliminate or drastically reduce inocula of endomycorrhizal fungi. Since spores of these fungi are not readily wind-disseminated like spores of ectomycorrhizal fungi, recolonization of the fumigated soil is slow and erratic. Poor growth of hardwoods in fumigated soil is at least partially caused by poor endomycorrhizal development. In 1974, we began research on the role of endomycorrhizae to growth of hardwoods. The research was patterned after the ectomycorrhizal fungus research. The purpose was to develop techniques for artificially infesting soil with specific endomycorrhizal fungi, for correcting deficiencies in fumigated soil in nurseries, and for tailoring seedlings for outplanting trials. Table 6 shows the results of the first test.[35] It is obvious from the data that sweetgum seedlings will not grow appreciably beyond the primary leaf stage without endomycorrhizae. This species has an obligate requirement for endomycorrhizae as Pinus has for ectomycorrhizae. These seedlings were grown in soil that received a total of 1120 kg/ha of calcium, 280 kg/ha of 10-10-10 fertilizer and 1568 kg/ha of NH_4NO_3. The latter was applied two times during the growing season at a rate of 784 kg/ha. Even with this ample soil fertility, the nonmycorrhizal seedlings were unable to obtain sufficient nutrients for growth. Seedlings with Glomus mosseae endomycorrhizae were over 32 times larger than nonmycorrhizal seedlings. Those with endomycorrhizae formed by a mixture of naturally occurring fungi (natural soil) were 54 times larger (total dry weight) than the nonmycorrhizal seedlings. These results strongly indicate that problems in production of quality sweetgum seedlings in nurseries are caused by a deficiency of endomycorrhizal fungi brought about by soil fumigation. The research was expanded in the spring of 1975 to include other hardwood species. Green and white ash, boxelder, and black cherry were tested in fumigated soil infested with different endomycorrhizal fungus inocula (unpublished data). Even though the seedlings in noninfested soil developed endomycorrhizae by midyear from inocula in soil below the effective fumigation zone (20 to 25 cm), they were significantly smaller than seedlings in artificially infested soil (Table 7). The data also indicate that certain endomycorrhizal fungi may stimulate hardwood seedling growth more than others. The inadvertent development of endomycorrhizae on the control seedlings in this test is probably similar to that which occurs in commercial nurseries. Poor seedling growth early in the growing season followed by acceptable growth beginning in midseason is probably related to root penetration and subsequent endomycorrhizal development in the zone below effective soil fumigation in the nursery bed. Acceptable growth of seedlings in mid-

season would follow this development of endomycorrhizae from inoculum surviving fumigation.

TABLE VI

Growth of 6-month-old sweetgum seedlings with and without endomycorrhizae.[1]

	Control	Glomus mosseae	Natural Soil
Height (cm)	5.5a	35.5b	44.2c
Root collar diameter (cm)	0.2a	0.7b	0.9c
Oven-dry root weight (gm)	0.2a	5.0b	8.5c
Total oven-dry weight (gm)	0.4a	13.3b	22.0c
Percent endomycorrhizae	0	100	100

[1] Means for mycorrhizal treatments sharing a common letter are not significantly different at the 0.5% confidence level.

TABLE VII

Growth of seedlings of four hardwood species after five months in fumigated soil artificially infested with different endomycorrhizal fungus inocula.[1]

Tree species	Mycorrhizal fungus inocula	Height (cm)	Stem dia. (cm)
Green Ash	Glomus mosseae	60.6a	1.2a
	G. fasciculatus	83.2b	1.6b
	Natural soil	79.4ab	1.5b
	Control[2]	38.2c	0.9c
White Ash	Glomus mosseae	21.9a	0.5a
	G. fasciculatus	37.2b	0.8b
	Natural soil	37.8b	0.8b
	Control[2]	9.4c	0.2c
Boxelder	Glomus mosseae	40.4a	1.0ab
	G. fasciculatus	57.9b	1.2a
	Natural soil	44.4a	1.0ab
	Control[2]	30.6c	0.8b
Black Cherry	Glomus mosseae	28.5a	0.5a
	G. fasciculatus	36.9b	0.6a
	Natural soil	34.1b	0.5a
	Control[2]	6.9c	0.2b

[1] Means for endomycorrhizal treatments of a tree species sharing a common letter are not significantly different at the 0.5% confidence level.

[2] All control seedlings developed endomycorrhizae in the third month from inocula in soil below effective fumigation.

7

PERFORMANCE OF SEEDLINGS WITH
SPECIFIC ENDOMYCORRHIZAE ON ADVERSE SITES

In one of our first field studies on hardwood endomycorrhizae, kaolin spoils in Georgia were planted in 1974 (unpublished data). Sycamore with and without endomycorrhizae formed by *Glomus mosseae* were grown in our experimental nursery in Athens. The seedlings were outplanted, and 60 kg/ha of 10-10-10 were broadcast yearly. Results after two years were significant (Table 8). Endomycorrhizae significantly improved survival on both sites and improved plot volume by 200 percent on the Fuller's earth spoil and 50 percent on the kaolin spoil. *Glomus mosseae* persisted very well on roots of the seedlings. However, after two years all sampled seedlings, even those initially nonmycorrhizal at planting, had many endomycorrhizae formed by indigenous fungi.

TABLE VIII

Survival and growth of sycamore seedlings after two years on strip-mined clay spoils in Georgia. Seedlings were grown with and without endomycorrhizae formed by *Glomus mosseae* in the nursery prior to outplanting.

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	PVI (cm^3)[1] 100
. . Normal Kaolin Spoil . .				
G. mosseae	49*	97.8	1.95	55*
Nonmycorrhizal	26	104.1	2.09	36
. . Fuller's Earth Spoil . .				
G. mosseae	62*	50.5*	0.97*	9*
Nonmycorrhizal	43	39.1	0.74	3

[1] Plot volume index (PVI) = (stem diameter)^2 X height X number of surviving trees (30 trees/replicate).

*Denotes significant differences (P = .05) between means for mycorrhizal conditions.

Another study (unpublished data) was installed on the same spoils in the spring of 1975. It involved sweetgum seedlings with and without *G. mosseae* endomycorrhizae. When planted, these seedlings were approximately the same size as those tabulated in Table 6. The stunted, nonmycorrhizal seedlings were planted to determine their survival and growth potential as well as to use their root systems as "traps" for determination of indigenous endomycorrhizal fungi in the spoils. The survival and growth after two years was strongly influenced by the initial endomycorrhizal condition (Table 9). Endomycorrhizae increased PVI by 15 times on the normal kaolin spoil and 12 times on the Fuller's earth spoil. Even though the nonmycorrhizal sweetgum seedlings were extremely small at planting they survived well. They only began to grow appreciably, however, after unidentified fungi formed endomycorrhizae on their roots about the middle of the first year on site.

Since some endomycorrhizal infection is apparent on wild grasses and volunteer trees on spoil material, it is logical to ask about the source of the initial inoculum. As mentioned, endomycorrhizal fungi do not have highly effective means of dissemination. They are usually spread by moving water or soil, insects, birds, and perhaps animals (including man). Their presence on strip-mined lands may be accounted for through

contamination of overburden material with the original topsoil. If some plants are dependent on, or at least stimulated by, endomycorrhizal infection, then increasing the amount of available inoculum in spoils might enhance performance of these plants on spoils. Tailoring these plants in nurseries or in containers with specific endomycorrhizal fungi also should be thoroughly tested.

In research on the value of endomycorrhizae to survival and growth of plants on strip-mined lands, testing the fungi that persist in the spoil should be emphasized. Perhaps an ecologically adapted endomycorrhizal fungus can be found which will have a similar potential to that of the ectomycorrhizal fungus *Pisolithus tinctorius*. Schenck and others[36] and Schenck and Schroder[37] have shown that some species of *Glomus* are more adapted to high soil temperatures than others. This suggests the possibility of selection among endomycorrhizal fungi for those species ecologically adapted to specific adverse sites having high soil temperatures. Techniques similar to those developed for ectomycorrhizal fungi are currently being developed in Athens for large scale artificial soil infestation with specific endomycorrhizal fungi.

TABLE IX

Survival and growth of sweetgum seedlings after two years on strip-mined clay spoils in Georgia. Seedlings were grown with and without endomycorrhizae formed by *Glomus mosseae* in the nursery prior to outplanting.

Mycorrhizal condition at planting	Percent survival	Height (cm)	Stem dia. (cm)	PVI (cm^3) 100
. . Normal Kaolin Spoil . .				
G. mosseae	98*	114.4*	2.1*	49*
Nonmycorrhizal	70	60.0	0.9	3
. . Fuller's Earth Spoil . .				
G. mosseae	100*	94.9*	1.7*	27*
Nonmycorrhizal	86	45.8	0.7	2

[1] Plot volume index (PVI) = (stem diameter)^2 X height X number of surviving trees (10 trees/replicate).

* Denotes significant differences (P = 0.05) between means for endomycorrhizal condition on a spoil.

ECTOMYCORRHIZAE
AND REFORESTATION OF ROUTINE SITES

Approximately 50,000 seedlings of various pine species with specific ectomycorrhizae have been experimentally outplanted in Georgia, Florida, North Carolina, South Carolina, Louisiana, Alabama, Arkansas, Mississippi, and Oklahoma. *Pisolithus tinctorius* is the major fungus used in these tests. The oldest available results are two-year data from four test sites in Florida and North Carolina. The seedlings planted on two sites in Florida were grown at the state nursery in Chiefland, Florida; seedlings planted on two sites in North Carolina were grown at the state nursery in Morganton, North Carolina.

These seedlings were part of an earlier nursery test.[24] Seedlings were graded to approximately equal heights and stem diameters before outplanting. The ectomycorrhizal treatments in the nursery were vegetative

mycelial inoculum (VMI) and basidiospore inoculum (BI)
of *P. tinctorius* and no inoculum (NI). All seedlings
had approximately 65 percent of their feeder roots ecto-
mycorrhizal at planting; however, those seedlings from
the VMI and BI treatments of *Pisolithus* had at least 75
percent and 35 percent of these ectomycorrhizae formed
by *P. tinctorius* and those from NI treatment had only
naturally occurring (primarily *Thelephora terrestris*)
ectomycorrhizae. The test sites near Brooksville,
Florida, were a deep sand ridge and a palmetto flatwood.
Both sites were planted to loblolly and slash pines and
the Ocala variety of sand pine. The sand ridge site
was considered fair to good for loblolly and slash pines
and good for sand pine; the palmetto flatwood site was
considered poor for loblolly and sand pines and good for
slash pine in Florida. In North Carolina, the sites
near Morganton were a clay loam hill with 25 cm of top
soil and an eroded slope of the same hill with little
topsoil. The former site was considered good for lob-
lolly, Virginia and white pines, and the latter site
poor for loblolly and Virginia pines in North Carolina.
White pine was only planted on the better site. After
two years, seedlings with the greatest quantity of
Pisolithus ectomycorrhizae at planting survived and grew
faster than seedlings with natural ectomycorrhizae.
Seedlings with less *Pisolithus* ectomycorrhizae (i.e.,
from BI in nursery) at planting were generally interme-
diate in survival and growth (unpublished data). For
this discussion only the VMI and NI treatments will be
compared using the PVI growth parameter.

Loblolly pine seedlings with *Pisolithus* ectomycorrhizae
on the good sites in North Carolina and Florida had
greater plot volumes than seedlings with natural ecto-
mycorrhizae, but these differences were even greater on
the poor sites (Figure 2). The plot volumes of seedlings
with *Pisolithus* ectomycorrhizae on the poor sites were
nearly identical to those of seedlings with natural
ectomycorrhizae on the good sites for loblolly pines in
both states. Both sites in Florida were good for slash
pine (Figure 3). The PVI's of seedlings with *Pisolithus*
ectomycorrhizae on both sites were about 80 percent
greater than seedlings with natural ectomycorrhizae.
These two sites in Florida had very strong effects on
sand pine, however (Figure 3). Sand pine seedlings with
Pisolithus ectomycorrhizae had a 270 percent greater
plot volume than seedlings with natural ectomycorrhizae
on the good site, and nearly a 450 percent greater plot
volume on the poor site. Also significant is the fact
that sand pine seedlings with *Pisolithus* ectomycorrhi-
zae on the poor site had a plot volume of over twice
that of seedlings with natural ectomycorrhizae on the
typical good sand pine site. Figure 4 shows the in-
fluence of *Pisolithus* ectomycorrhizae on seedlings of
Virginia and white pines in North Carolina. As with
the other pine species, *Pisolithus* ectomycorrhizae on
Virginia pine caused greater differences in plot volumes
on the poor site (55 percent) than on the good site
(30 percent). However, plot volumes were similar be-
tween seedlings with *Pisolithus* ectomycorrhizae on the
poor site and seedlings with natural ectomycorrhizae on
the good site. Only one site was planted to white pine
seedlings in North Carolina, but the results were
dramatic (Figure 4). White pine seedlings (1-0) with
Pisolithus ectomycorrhizae had 500 percent greater plot
volume than seedlings (1-0) with natural ectomycorrhizae.

Root evaluation for *Pisolithus* ectomycorrhizae on seed-
lings in the above tests showed that *Pisolithus* per-
sisted extremely well on these sites, in spite of com-
petition from other ectomycorrhizal fungi that were in-
digenous on these sites. However, the more *Pisolithus*
ectomycorrhizae there were on roots at planting the
greater the persistence and, as previously discussed,
the greater the growth increases on seedlings.
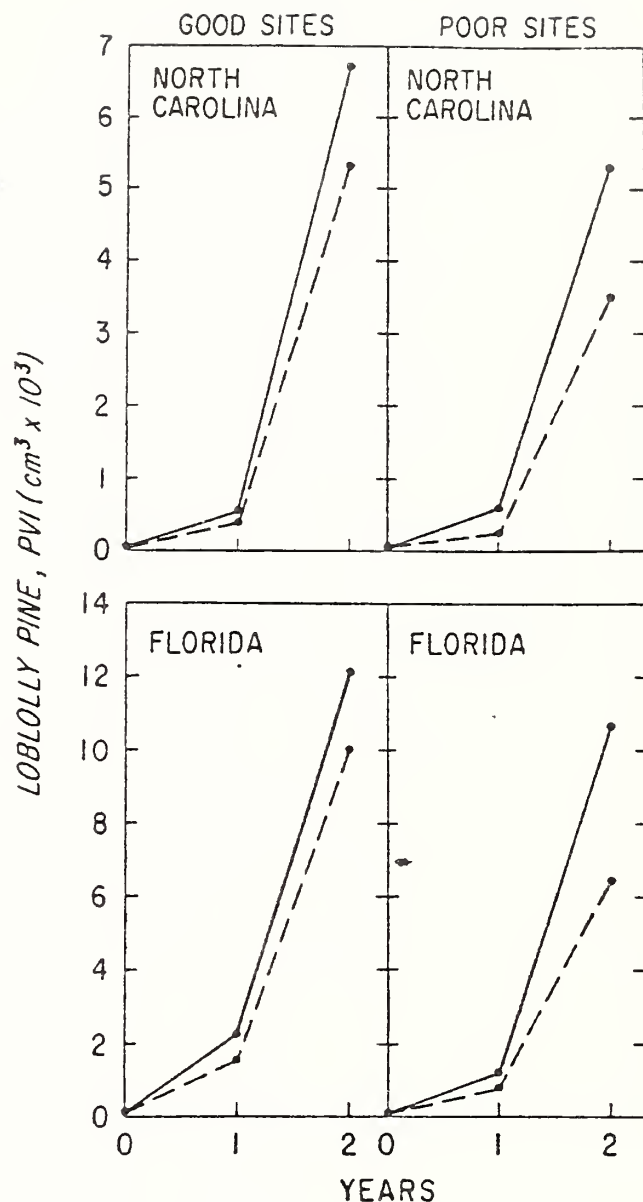
FIGURE 2. Plot volume indices (PVI) of loblolly pine
seedlings with *Pisolithus* (●——●) or natural (●--●) ecto-
mycorrhizae at planting on good and poor sites in
North Carolina and Florida. PVI = (stem diameter)^2
X height X number of surviving seedlings per plot.

CONCLUSIONS

The manipulation of both ectomycorrhizal and endomycor-
rhizal fungi in nurseries or in container programs may
be the biological tool needed to improve reclamation
and reforestation efforts throughout the world. At
this time, only one ectomycorrhizal fungus--*Pisolithus
tinctorius*--is approaching a practical application
stage. However, its successful manipulation in
nurseries and its subsequent impact on tree performance
on a variety of sites shows the potential practical
importance of these highly specialized, root-inhabiting
fungi in forestry. When one considers the vast species
of mycorrhizal fungi in nature that may be manipulated
it is exciting to contemplate their potential impact on
forest production throughout the world.
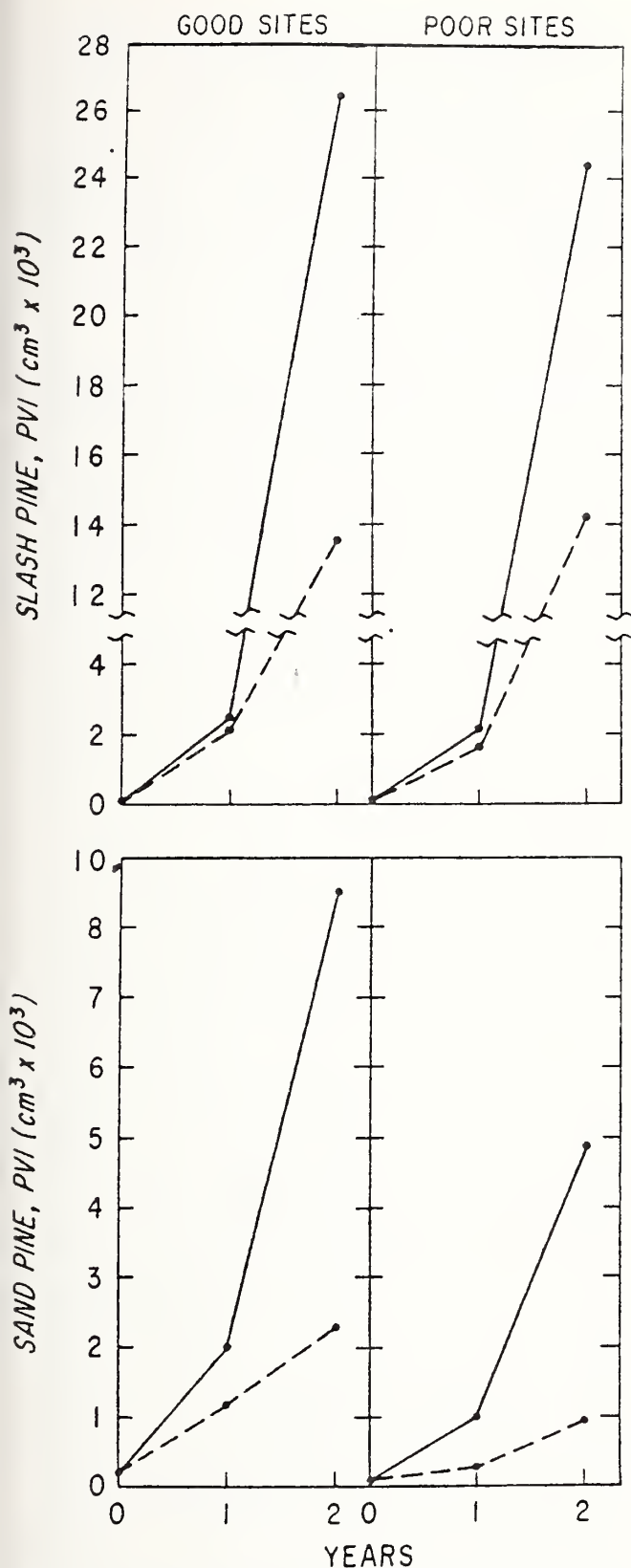
FIGURE 3. Plot volume indices (PVI) of slash and sand pine seedlings with *Pisolithus* (———) or natural (-----) ectomycorrhizae at planting on good and poor sites in Florida. PVI = (stem diameter)^2 X height X number of surviving seedlings per plot.
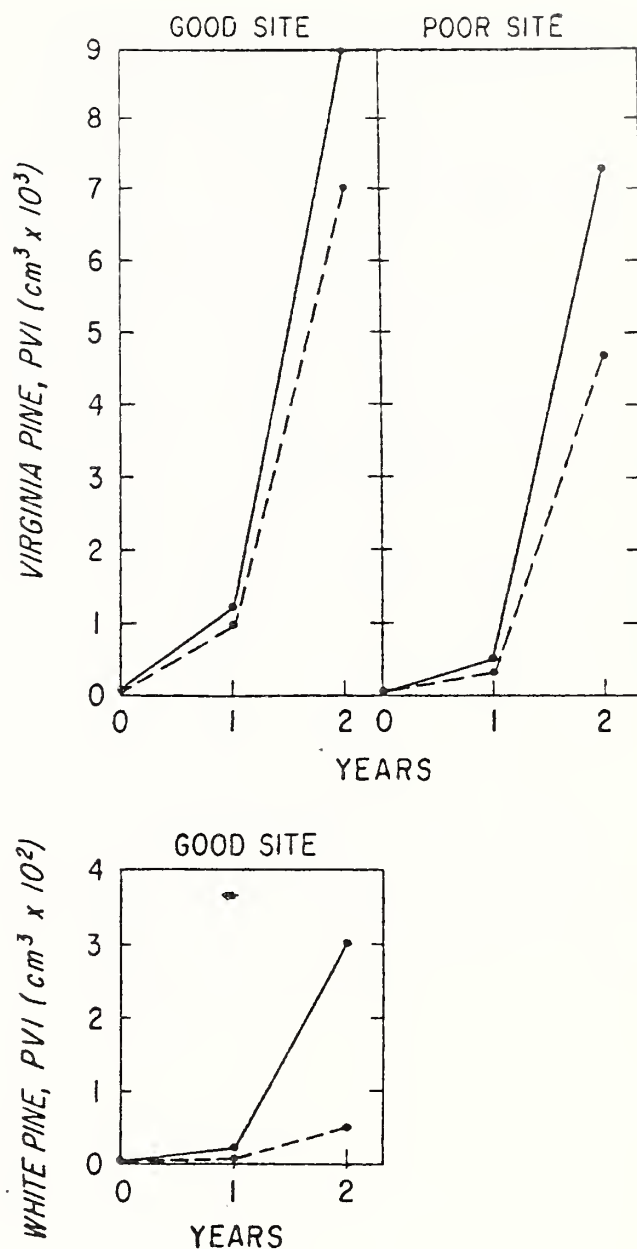
FIGURE 4. Plot volume indices (PVI) of Virginia pine seedlings with *Pisolithus* (———) or natural (-----) ectomycorrhizae on a good and poor site, and white pine on a good site in North Carolina. PVI = (stem diameter)^2 X height X number of surviving seedlings per plot.

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DEELY

Variability in ectomycorrhizal development and growth among isolates of *Pisolithus tinctorius* as affected by source, age, and reisolation

DONALD H. MARX

(Theodorou and Bowen 1970). These authors and others (Melin 1936; Trappe 1977; Shemakhanova 1967; Gobl 1975) have emphasized the need to use fresh isolates (recently obtained in pure culture) and to test several isolates before deciding on the merits of individual fungal species.

Pisolithus tinctorius (Pers.) Coker and Couch is a fungus which has potential for practical application in forestation programs. Pine seedlings with *Pisolithus* ectomycorrhizae synthesized in nurseries survive and grow faster than those with other ectomycorrhizae on adverse (Marx and Artman 1979) and routine reforestation sites (Marx *et al.* 1977) in the southern United States. This fungus also has a broad tree-host range and is distributed throughout most of the world (Marx 1977). Variability among isolates of *P. tinctorius* in growth rate in pure culture, and in ectomycorrhizal development on oak in fumigated nursery soil (Marx 1979a), containerized Douglas-fir, and lodgepole pine (Molina 1979), and in aseptic synthesis test on loblolly pine (Marx *et al.* 1970) has been reported. Before *P. tinctorius* can be fully exploited for practical use in forestry, variability among isolates must be more fully defined.

Various disease studies have shown that repeated passage of a pathogen through susceptible plant hosts enhances virulence of fungal pathogens (Day 1960). Host passage apparently affects the ability of the fungus to produce sufficient quantities of adaptive enzymes in response to stimulation from the enzyme substrates produced by the plant host. Extended periods of vegetative growth on artificial media in the absence of essential substrates and without frequent reassociation with susceptible plant tissue may change adaptive enzyme systems and decrease the ability of the fungus to infect host tissue. Ectomycorrhizal fungi also utilize specific enzymes to infect host roots (Hacskaylo 1973) and many lose their ability to form ectomycorrhizae after extended culture on agar media (Marx and Daniel 1976). Perhaps frequent passage of ectomycorrhizal fungi through susceptible tree hosts can revitalize (increase ectomycorrhizal development of) older cultures that have lost symbiotic capacities after extended periods of time on synthetic media. Frequent host passage may also maintain original vitality of the isolates. This procedure was used on ectomycorrhizal fungi after aseptic synthesis tests by Melin (1936), but is rarely used by contemporary researchers of ectomycorrhizal fungi.

The purpose of this research was to examine the growth and ectomycorrhizal development of diverse isolates of *P. tinctorius* obtained from different tree hosts and geographic sources and of various ages and to determine whether passage (ectomycorrhizal synthesis and reisolation) through *Pinus taeda* seedlings affects

the capacity of the isolate to form ectomycorrhizae on pine.

Materials and methods

Maintenance of isolates

Twenty-one isolates were used (Table 1). All cultures were confirmed free of bacterial contamination (Marx 1979a). They were grown for 2 to 4 weeks in test tube slants of modified Melin – Norkrans (MMN) medium with glucose (Marx 1969) and stored at 5°C in darkness. Isolates were subcultured on MMN agar medium every 1 to 2 years and stored at 5°C.

Inoculum preparation

To prepare an inoculum for ectomycorrhizal synthesis, the isolates were removed from storage and grown on plates of MMN agar for 30 days. Each isolate was then grown in vermiculite – peat moss – nutrient medium for 3 months at 21 – 27°C. The inoculum was removed from the containers and leached in tapwater; excess water was removed by hand pressure (Marx and Bryan 1975). The inoculum was stored at 5°C for a maximum of 3 days before use.

Initial synthesis of ectomycorrhizae on pine

The initial test for ectomycorrhizal synthesis was conducted in an electronically air-filtered, air-conditioned growth room (Marx 1973). Air filtration excludes, among other microorganisms, naturally occurring ectomycorrhizal fungi disseminated by air-borne spores, thereby eliminating competition to the test fungi.

The soil mixture (1 : 1, v : v, sand and forest clay loam) and 20-cm-diameter clay pots were steamed three times on alternate days for 4 h at 85°C. A vermiculite – mycelium inoculum of each isolate was mixed 1 : 6 by volume with the soil mixture in the growth room. Ten pots, each containing 2 cm of steamed gravel to facilitate drainage, were filled with soil infested with an isolate. Control soil contained no fungus. Commercial 10 – 10 – 10 fertilizer was mixed (500 kg/ha) into the upper 10 cm of soil in each pot. Seeds were presoaked for 48 h at 5°C in 1% H_2O_2 to stimulate germination. Each pot was sown with seeds of *P. taeda* (Georgia piedmont source), and after 3 weeks seedlings were thinned to one per pot. Pots were arranged on a bench in the growth room in a randomized block design having 22 fungal treatments (21 isolates and a control) with 10 seedlings each. Soil was infested and seeds were sown in late February 1976. Seedlings received approximately 75% of full sunlight filtered through the fiber glass covering of the growth room for approximately 13 h daily. Day and night air temperatures in the growth room averaged 26 and 20°C, respectively. Seedlings were watered as needed.

After 5 months, seedlings were removed from the pots and soil was washed from roots. Ectomycorrhizal development on each seedling was visually estimated without magnification (Marx and Bryan 1975). Representative ectomycorrhizae were freehand sectioned, mounted in phloxine – lactophenol, and examined for fungal mantle and Hartig-net characteristics at 100×. No seedling measurements were recorded and all seedlings were healthy.

Reisolation from ectomycorrhizae

Lateral roots (2-cm lengths) with *P. tinctorius* ectomy-

TABLE 1. History, age, and ectomycorrhizal development of different isolates of *Pisolithus tinctorius* on loblolly pine seedlings in growth room

Isolate No.	Tree host	Collection site	Collection location	Isolate obtained by	Years in pure culture	% ectomycorrhizal development[a]
29	*Pinus echinata*	Forest	Georgia	Zak, B.	17	<2 (1)
98	*P. virginiana*	Coal spoil	Kentucky	Author	6	21−50 (8)
106	*P. virginiana*	Coal spoil	Kentucky	Author	6	21−50 (6)
110	*P. taeda*	Coal spoil	Kentucky	Author	5	>51 (10)
121	*P. taeda*	Forest	Georgia	Otrosina, W.	3	>51 (10)
124	*P. echinata*	Forest	Georgia	Author	3	>51 (10)
125	*P. taeda*	Nursery	North Carolina	Author	3	>51 (9)
135	*Pinus* sp.	Forest	Taiwan	Davidson, R.	2	>51 (10)
136	*Quercus palustris*	Urban	Georgia	Author	2	0 (0)
138	*P. taeda*	Forest	Georgia	Author	1	>51 (10)
142	*P. taeda*	Coal spoil	Virginia	Author	2	>51 (9)
145	*Q. acutissima*	Coal spoil	Kentucky	Author	2	3−20 (4)
152	*P. elliottii*	Forest	Australia	Lamb, R.	3	0 (0)
153	*P. elliottii*	Forest	Australia	Lamb, R.	6	0 (0)
155	*P. elliottii*	Forest	Australia	Lamb, R.	7	0 (0)
156	*P. patula*	Forest	Australia	Lamb, R.	4	0 (0)
157	*P. patula*	Forest	Australia	Lamb, R.	4	0 (0)
158	*P. strobus*	Coal spoil	Ohio	Leben, C.	2	>51 (10)
177	*Q. alba*	Urban	Virginia	Artman, J.	1	<2 (4)
179	*P. radiata*	Forest	Brazil	Hodges, C.	1	3−20 (4)
183	*P. pinaster*	Coal spoil	France	Guinberteau, J.	1	>51 (10)

[a]Number in parentheses is the number of seedlings with *Pisolithus* ectomycorrhizae out of the 10 tested.

corrhizae were placed in perforated plastic vials. These were exposed to a detergent wash (10 drops of detergent in 1 L of tap water) for 30 s to reduce surface tension. Vials were placed, without rinsing, directly into 100 ppm of $HgCl_2$ and agitated vigorously for 3 min, then rinsed in sterile water three times for 15 min each. From each isolate forming sufficient ectomycorrhizae, 100 individual ectomycorrhizae were placed singly in small tubes of MMN agar medium and incubated at room temperature. The ectomycorrhizae were examined twice weekly for 8 weeks for the distinctive gold−brown hyphae of *P. tinctorius*. Reisolated cultures were confirmed free of bacterial contaminants (Marx 1979a).

Growth in pure culture on agar medium

The original isolates and reisolates were grown in plates of MMN agar for 30 days at 25°C. Eight-millimetre-diameter discs of mycelium and agar were removed from the outer periphery of mycelial growth and used to inoculate plates of MMN agar (30 mL/plate). Eighteen plates of each culture were prepared and incubated at 25°C for 4 days to confirm growth from the inoculum disc. From each culture, six plates were incubated at each of 15, 25, and 35°C (±2°C). After 14 days, colony diameters were measured. Data were processed by analysis of variance, and significant differences among means were identified with Duncan's multiple range test at $P = 0.05$.

Test in experimental nursery

Eight original isolates and nine reisolates were tested for their ability to form ectomycorrhizae on *P. taeda* seedlings and influence growth in a microplot experimental nursery. The original isolate No. 138 was reisolated from pine, as were

the other isolates, but an additional reisolate of No. 138 was obtained from ectomycorrhizae formed earlier on pecan seedlings in this nursery (Marx 1979b). Both reisolates of No. 138 were the same age and were reisolated from ectomycorrhizae by identical procedures.

Ninety wood-frame microplots (0.6 × 0.6 × 0.6 m) were placed 1.5 m apart on level ground, filled to a depth of 0.2 m with gravel, and fumigated with methyl bromide. Soil mixture (2 : 1 : 1, by volume, of forest clay loam, sand, and milled pine bark) was fumigated on a concrete pad and then used to fill the microplots. All fumigation was done with Dowfume MC-2[3] (Dow Chemical Co., Midland, MI) at 1 kg/18 m² of soil surface (25 cm deep) under clear polyethylene plastic for 48 h. The soil mixture had a pH 5.2 and contained 8 ppm of available P, and 54, 120, 22, and 45 ppm of exchangeable K, Ca, Mg, and Mn, respectively. Soil contained 150 ppm of total N and 1.9% organic matter.[4]

Inoculum at a rate of 1.08 L/m² of soil surface and granulated commercial 10−10−10 fertilizer (290 kg/ha) were broadcast evenly over the surface of the microplots and mixed into the upper 12 cm of soil with hand tools. Fertilizer and leached vermiculite − peat moss − nutrient medium without fungus were similarly added to the soil of control plots.

[3]Trade or proprietary names are included for information purposes only and do not imply any endorsement by the Canadian Journal of Forest Research and the Forest Service of the United States Department of Agriculture.

[4]Soil analyses were conducted by Carol G. Wells, United States Department of Agriculture, Forest Service, Forestry Sciences Laboratory, Research Triangle Park, NC, 27709.

Microplots were arranged in a randomized block design with 17 isolate treatments and a control treatment; each treatment was represented in five blocks. Seed of *P. taeda* (Georgia piedmont source) were treated with Arasan®−latex sticker, planted in three 0.6-m-long rows on 15-cm centers in each microplot, and covered with 5 mm of soil. Fumigated pine straw was placed 2 cm deep over soil as a mulch. Microplots were watered as needed. Three weeks after germination, seedlings were thinned to approximately 100 per microplot (300 seedlings/m²).

The study was installed in April 1977. Seedlings were irrigated twice weekly and fertilized with 56 kg N/ha (as NH_4NO_3) in early July and again in early August. Beginning in late July, microplots were examined twice weekly and the incidence of fruit bodies of *P. tinctorius* were recorded.

In February 1978, seedlings were vertically cut between rows and undercut 20 cm deep with a shovel. Seedlings were removed by hand and roots washed in water. All seedlings were examined for the presence or absence of *P. tinctorius* ectomycorrhizae and graded as plantable or cull seedlings. Those less than 12 cm tall with root-collar diameters less than 2 mm or without secondary needles were considered culls and discarded; the remaining seedlings were considered plantable. Culling was done to eliminate suppressed seedlings which resulted from late-germinating seed; about 90% of the seedlings were from seed which germinated within 14 days of sowing. Ten plantable seedlings per microplot were selected at random and measured for height, root-collar diameter, and top and root fresh weights. Ectomycorrhizae were quantitatively assessed visually on the sample seedlings. Data on ectomycorrhizal development were converted into a *Pisolithus tinctorius* (Pt) index using the formula $a \times b/c$ where a = percent of seedlings with any amount of Pt ectomycorrhizae, b = average percent of feeder roots with Pt ectomycorrhizae (including 0% for seedlings without Pt), and c = average percent of total ectomycorrhizal development on seedlings formed by Pt and other fungi. An index of 100 indicates that all the ectomycorrhizae formed on all seedlings were formed by Pt. This index integrates into a single value all measurements on Pt ectomycorrhizal development which represent the aggressiveness of the isolate or the effectiveness of the inoculum. All data were analyzed as mentioned earlier.

Mycelium of all cultures of *P. tinctorius* completely colonized the vermiculite − peat moss − nutrient medium used as inoculum in the two synthesis tests. Differences in ectomycorrhizal development among cultures, therefore, were assumed due to inherent physiological differences and not to amount of mycelium in the original inoculum.

Results

Initial synthesis of ectomycorrhizae on pine

Isolate 136 from oak in Georgia and isolates 152, 153, 155, 156, and 157 from pine in Australia did not form any *Pisolithus* ectomycorrhizae on the loblolly pine seedlings in the growth room study (Table 1). No evidence of saprophytic growth, i.e., visible hyphal strands, of *P. tinctorius* was observed in soil or on roots of these seedlings. Isolates 28, 145, 177, and 179 formed ectomycorrhizae, but the incidence was very

low. Usually only 1 to 4 pine seedlings of the 10 seedlings tested had ectomycorrhizae. The other 11 isolates formed abundant ectomycorrhizae, usually on all 10 test seedlings. No obvious differences in color or morphology of the ectomycorrhizae formed by different isolates were observed. Most ectomycorrhizae were complex coralloid and mustard−yellow to gold−brown, with numerous, similarly colored hyphal strands radiating from the ectomycorrhizae into the soil. Fungus mantles varied in thickness from 12 to 48 μm and the Hartig-net hyphae usually penetrated to the endodermis.

Reisolation from ectomycorrhizae

Ten original isolates were reisolated from ectomycorrhizae on the pine seedlings. Isolates 29, 142, 145, and 179 were not reisolated in pure culture, either because of insufficient numbers of ectomycorrhizae or because of excessive microbial contamination. Isolate 124 was reisolated from its ectomycorrhizae but was inadvertently destroyed.

Growth in pure culture on agar medium

Three isolates at 15°C (98, 121, and 138) and 25°C (98, 121, and 177) and four isolates (98, 106, 110, and 138) at 35°C were significantly affected in growth rate by reisolation; some isolates grew faster and some grew slower (Table 2). There were large differences in growth rates between the original isolates at the different temperatures. All but one isolate (106), regardless of culture condition, grew better at 25 and 35°C than at 15°C.

Test in experimental nursery

Original isolates and reisolates of 138, 158, and 183 and reisolate 121 formed significantly more ectomycorrhizae (greater Pt indices) than did other isolates (Table 3). Original isolate 106 had a Pt index of 0 while its reisolate had an index of 44 indicating the value of host passage for this isolate. Reisolation also significantly improved Pt indices of isolates 98 and 121. Reisolation did not affect indices of the other five isolates, but as a group, the reisolates had greater Pt indices and produced more fruit bodies than the original isolates.

Total number of seedlings and number of cull seedlings were not affected by isolate treatment. Isolate 138 (all sources) was the only one to significantly increase seedling growth in comparison to control seedlings. Because of this, discrete seedling measurements are not presented. Test seedlings ranged in height from 20 to 23 cm, in root-collar diameters from 4 to 5 mm, and in total fresh weights from 10 to 13 g.

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TABLE 2. Mycelial colony diameters of different isolates of *Pisolithus tinctorius* in original and reisolated conditions on agar medium after 14 days at different temperatures[a]

Isolate No., host, and age	Isolate condition[b]	Mycelial colony diameter (mm)		
		15°C	25°C	35°C
98, *Pinus virginiana*, 7 years	Original	20.3*b*	34.5*b*	26.7*b*
	Reisolate	25.2*a*	43.2*a*	35.3*a*
106, *P. virginiana*, 7 years	Original	19.5*a*	40.8*a*	30.3*a*
	Reisolate	19.6*a*	43.2*a*	19.3*b*
110, *P. taeda*, 6 years	Original	24.8*a*	35.5*a*	47.7*a*
	Reisolate	16.7*a*	36.3*a*	35.7*b*
121, *P. taeda*, 4 years	Original	36.3*a*	78.5*a*	43.1*a*
	Reisolate	26.2*b*	58.2*b*	35.5*a*
125, *P. taeda*, 4 years	Original	16.8*a*	22.7*a*	20.5*a*
	Reisolate	11.5*a*	24.8*a*	25.2*a*
135, *Pinus* sp., 3 years	Original	15.1*a*	24.2*a*	21.6*a*
	Reisolate	14.3*a*	24.8*a*	22.2*a*
138, *P. taeda*,[c] revitalized	Original	21.2*b*	51.1*ab*	27.5*b*
biennially for 10 years	Reisolate pine	32.4*a*	60.6*a*	32.3*a*
	Reisolate pecan	26.2*ab*	49.5*b*	34.1*a*
158, *P. strobus*, 3 years	Original	21.2*a*	44.1*a*	33.6*a*
	Reisolate	25.6*a*	50.8*a*	30.2*a*
177, *Quercus alba*, 2 years	Original	22.8*a*	58.2*a*	40.5*a*
	Reisolate	19.2*a*	43.8*b*	38.4*a*
183, *P. pinaster*, 2 years	Original	22.4*a*	46.5*a*	41.6*a*
	Reisolate	18.7*a*	46.3*a*	37.5*a*
Overall isolate means	Original	22.0*a*	43.6*a*	33.3*a*
	Reisolate	21.4*a*	43.8*a*	31.4*a*

[a]Each number is the mean of six replicate plate cultures. For each isolate, means followed by a common letter are not significantly different at $P = 0.05$.

[b]Original isolates were maintained in storage on agar medium at 5°C; reisolates were obtained from ectomycorrhizae formed by the original isolate on loblolly pine seedlings in growth room.

[c]Only 1 year had elapsed since the original No. 138 was last reisolated. Original in this instance does not mean isolate in agar storage for 10 years. Reisolates were obtained from pine ectomycorrhizae formed in growth room and from pecan ectomycorrhizae formed in microplot.

oak isolate from the United States and five pine isolates from Australia and the low incidence of ectomycorrhizae formed by the other oak isolates and the Brazilian pine isolate indicate that host and world location play significant roles in variation in *Pisolithus tinctorius*. Age in pure culture of these isolates was apparently not responsible for the lack of symbiotic potential, as other isolates much older and from different hosts and locations formed abundant *Pisolithus* ectomycorrhizae. The abundant *Pisolithus* ectomycorrhizae formed by isolate 135 from Taiwan and 183 from France in all tests indicates that differences in world location do not always affect symbiotic potential on *P. taeda*. Apparently, however, those isolates near or south of the Tropic of Cancer have different potentials on *P. taeda* than those north of this zone. This supposition needs considerable more research before it can be validated. Age of pure culture, and undoubtedly, conditions during culture storage, are important, however. The 17-year-old pine isolate from the United States formed very few ectomycorrhizae on pine in this study, whereas earlier this isolate formed abundant mycorrhizae (Marx *et al.* 1970).

Reisolation from ectomycorrhizae formed by original isolates did not consistently influence growth rate in pure culture; many reisolates grew slower than their originals. Furthermore, there were no consistent relationships between rate of growth in pure culture and degree of *Pisolithus* ectomycorrhizal development.

Revitalization of 4-year-old or older pine isolates via host passage and reisolation was successful. Evidence for this conculsion is that, as a group, the reisolates formed significantly more *Pisolithus* ectomycorrhizae and fruit bodies than the original isolates. However, host passage did not improve symbiotic potential of all isolates. It would be interesting to determine if repeated passages through pine would make the oak isolates, which were poor in pine, more efficient on pine. These oak isolates were poor symbionts on oak in an earlier study (Marx 1979a).

TABLE 3. Ectomycorrhizal development of loblolly pine seedlings from fumigated soil in microplots artificially infested with different original isolates and reisolates of *Pisolithus tinctorius* (Pt)[a]

Isolate No., host, and original age	Isolate condition	% ectomycorrhizae by[b]		% seedlings with Pt[c]	Pt index[d]	No. of Pt fruit bodies[c]
		Pt	total			
98, *Pinus virginiana*, 7 years	Original	10c	30c	30c	9c	0c
	Reisolate	23bc	38c	88b	56b	1b
106, *P. virginiana*, 7 years	Original	0d	31c	0d	0	0c
	Reisolate	22bc	37c	69b	44b	2c
121, *P. taeda*, 4 years	Original	27bc	48bc	100a	55b	0c
	Reisolate	68a	70a	100a	93a	2b
135, *Pinus* sp., 3 years	Original	8c	37c	56b	14c	0c
	Reisolate	8c	36c	55b	12c	0c
138, *P. taeda*, revitalized biennially for 10 years	Original	58a	64a	100a	90a	2b
	Reisolate pine	58a	64a	100a	86a	1b
	Reisolate pecan	52a	61a	100a	84a	0c
158, *P. strobus*, 3 years	Original	53a	65a	100a	80a	6b
	Reisolate	57a	65a	100a	86a	18a
177, *Quercus alba*, 2 years	Original	4c	34c	34bc	3c	0c
	Reisolate	5c	34c	47b	8c	0c
183, *P. pinaster*, 2 years	Original	42b	53b	100a	77a	4b
	Reisolate	48b	58b	100a	83a	4b
Control		0d	31c	0d	0d	0c
Mean of all isolates	Original	25	45	65	41	12
	Reisolate	38*	51	84*	61*	26*

[a]Within a column, means followed by the same letters are not significantly different at $P = 0.05$, * denotes significant difference at $P = 0.05$ for means of all isolate data.

[b]Mean from 10 randomly selected seedlings from each of five microplots per isolate and condition. All seedlings had some ectomycorrhizae formed by naturally occurring fungi in addition to those formed by Pt.

[c]Mean from five microplots per treatment.

[d]Pt index computed by $a \times b/c$ where a = percent of seedlings with any amount of Pt ectomycorrhizae, b = average percent of Pt ectomycorrhizae on sampled seedlings (including 0% for those without Pt), and c = average percent of all ectomycorrhizae formed by Pt and other fungi.

Isolate 138 was the only isolate to stimulate seedling growth and to consistently form abundant ectomycorrhizae in these tests. For the past 10 years, this isolate has been passed repeatedly through pine hosts. This repeated host passage may explain its superiority over other isolates in these tests and those with oak (Marx 1979a), Douglas-fir, and lodgepole pine (Molina 1979).

Even though older isolates or those from different hosts may not have the proper quantity or sufficient activity of essential adaptive enzymes needed to form abundant ectomycorrhizae on loblolly pine, other factors must also be considered. The test inoculum was exposed to various conditions from the time of its aseptic production to its exposure to roots in fumigated or steamed soil. Those isolates that demonstrated low symbiotic potential may not be able to survive mechanical manipulation during inoculum processing and incorporation into soil. Perhaps they cannot survive microbial competition in the soil. They may be excellent symbionts under natural conditions but poor symbionts under these experimental conditions.

It can be concluded from this study that isolates of *P. tinctorius* that have been (1) in actively growing cultures on agar media for over 4 years, (2) collected from oak hosts, or (3) obtained from pine hosts south of the Tropic of Cancer should be thoroughly tested before use in basic or applied research on loblolly pine. Any of these variables could be responsible for significant variation resulting in poor ectomycorrhizal development on loblolly pine. Revitalization via host passage should be done at least every 4 years to maintain a high level of symbiotic potential for specific isolates.

Because *Pisolithus tinctorius* and other ectomycorrhizal fungi exhibit considerable variation, several isolates representing different sources of potential variation (age, tree host, world location) should be examined before the symbiotic potential of any fungus species can be properly evaluated.

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Pisolithus tinctorius Ectomycorrhizae Improve Survival and Growth of Pine Seedlings on Acid Coal Spoils in Kentucky and Virginia

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Nursery grown seedlings of loblolly (*Pinus taeda*) and shortleaf pine (*Pinus echinata*) with ectomycorrhizae formed by *Pisolithus tinctorius* survived and grew significantly better than seedlings with *Thelephora terrestris* ectomycorrhizae after 3 years on an acid coal spoil in Kentucky and 4 years on an acid coal spoil in Virginia. Seedlings with *Pisolithus* ectomycorrhizae on the Kentucky spoil had significantly more N and less S, Fe, Mn, and Al in needles than seedlings with *Thelephora* ectomycorrhizae after 3 years. Seedlings with *Thelephora* ectomycorrhizae were more susceptible to winter injury on both spoils. On the Kentucky spoil, slow-release nutrients from fertilizer tablets significantly increased seedling growth for the first 2 years, but after nutrients were depleted, seedlings exhibited N-deficiency symptoms. After 3 years, nonfertilized seedlings of *P. taeda* with *Pisolithus* ectomycorrhizae were larger than seedlings with *Thelephora* ectomycorrhizae and fertilizer tablets. The ability of *P. tinctorius* to persist and spread to new roots stimulated seedling growth on these acid spoils and the lack of persistence and spread of *T. terrestris* accounted for poor seedling survival and growth.

THE IMPORTANCE of ectomycorrhizae to the growth and development of pine in natural forest soils is well known. Many different species of fungi, mainly Basidiomycetes which produce mushrooms or puffballs, form ectomycorrhizae on roots of pine in the normal forest soil environment (Marks and Kozlowski, 1973). Certain of these fungi appear to be better adapted to extremes of soil conditions, such as pH, organic matter, moisture, and temperature, than are other fungal species (Trappe, 1977). Under stress conditions, some species of fungi survive and grow better on roots than others and, therefore, are more beneficial to their tree hosts. Many workers (Schramm, 1966; Medve et al., 1977; Marx, 1977a; Hile and Hennen, 1969; Meyer, 1968; Lampky and Peterson, 1963) have ob-

served the widespread occurrence of sporophores of *Pisolithus tinctorius* under numerous tree species growing on acid coal spoils in various parts of the world. Usually only a few other species of ectomycorrhizal fungi occur under such adverse conditions. Marx (1977b) reported *Pisolithus* associated with trees growing on other such adverse sites, such as kaolin spoils and severely eroded sites in the southeastern United States. Pines need ectomycorrhizae to grow in natural situations and since *Pisolithus* is commonly associated with pines growing on acid coal spoils and other adverse sites, it is logical to assume that *Pisolithus* ectomycorrhizae contribute significantly to the health and vigor of seedlings on these sites.

Nursery grown pine seedlings are used in many reclamation programs in the eastern United States. Following fumigation of nursery soils, seedlings become ectomycorrhizal with *Thelephora terrestris*, a fungus particularly adaptable to the high fertility and moisture normally found in nurseries. It is spread naturally via windblown spores. Pine seedlings with *Thelephora* ectomycorrhizae do not survive or grow well at high soil temperatures encountered during summer months on coal spoils; seedlings with *Pisolithus* ectomycorrhizae, however, survive and grow well at high soil temperatures (Schramm, 1966; Marx, Bryan and Davey, 1970; Marx and Bryan, 1971). The generally poor performance of nursery seedlings after outplanting on many coal spoils may be partially due to the fact that the fungal symbiont on their roots at planting cannot tolerate the adversity of the coal spoil. Seedlings that do survive usually begin to grow rapidly following the natural but erratic appearance of *Pisolithus* ectomycorrhizae on root systems (Schramm, 1966; Marx 1977a). *Pisolithus* occurs at random from windblown basidiospores released from sporophores produced in adjacent forests. The above observations indicate that survival and growth of pine seedlings on acid coal spoils may be improved if *Pisolithus* ectomycorrhizae were formed on the seedlings in the nursery prior to their outplanting on the spoils.

Experimental procedures have been developed to artificially infest fumigated nursery soils with pure cultures of *P. tinctorius* in order to form abundant *Pisolithus* ectomycorrhizae on pine seedlings (Marx and Bryan, 1975; Marx, Bryan and Cordell, 1976; Marx and Artman, 1978; Marx, Morris and Mexal, 1978). Two-year field tests on routine reforestation sites in North Carolina and Florida revealed that pine seedlings with abundant *Pisolithus* ectomycorrhizae survived and grew better than seedlings with fewer *Pisolithus* ectomycorrhizae, or seedlings having only *Thelephora* ectomycorrhizae. Growth differences of 25 to 450 percent were observed on different pine species in certain of these comparisons (Marx, Bryan and Cordell, 1977). It became apparent from field studies that as site quality decreased, the value of *Pisolithus* ectomycorrhizae to the seedlings increased. *Pisolithus* maintained its dominance on seedling roots on the poorer sites but other ectomycorrhizal fungi were also frequently observed on seedlings on the better quality sites after 2 years. In other tests on the severely eroded sections of the Copper Basin of Tennessee, seedlings of *Pinus virginiana* and *P. taeda* with *Pisolithus* ectomycorrhizae were nearly twice as large after two years as seedlings with only *Thelephora* ectomycorrhizae (Berry and Marx, 1978). Both fungi persisted on seedling roots very well on this site, apparently because few other competitive ectomycorrhizal fungi were present.

The studies reported here were designed to determine the significance of *P. tinctorius* ectomycorrhizae to survival and growth of pines on coal spoils in Kentucky and Virginia. Since several workers (Zarger et al., 1973) have shown the value of fertilizer to growth of trees on coal spoils, a fertility variable was also tested on the Kentucky spoil. Slow release fertilizer, in the form of seedling starter-tablets, was used because they have been shown to benefit early growth of tree seedlings on borrow pits (White, 1963) and routine reforestation sites (Austin and Strand, 1960; Meskimen, 1971).

MATERIALS AND METHODS

Kentucky Coal Spoil

Seedling Production: Mycelial inoculum of *P. tinctorius* (isolate 126) was grown for 3 months in 2 liter containers and leached (Marx and Bryan, 1975). The inoculum was broadcast at a rate of 1.6 l/m² of soil surface with 560 kg/ha of 10- 10- 10 fertilizer onto recently fumigated nursery soil and mechanically incorporated 12 cm into the soil. Autoclaved inoculum was similarly prepared and added to soil for production of control seedlings which became ectomycorrhizal with naturally occurring *T. terrestris*. The soil, a 2:1:1 volume mixture of forest clay loam: sand: milled pine bark, contained in 3 m×1 m×0.3 m deep, wood-frame plots was fumigated with recommended rates of methyl bromide. Seeds of *P.*

taeda (North Carolina seed source) and *P. echinata* (northern Georgia seed source) were coated with Arasan® and late sticker and planted in April 1974 in rows 10 cm apart in the plots. Seed of each pine species were planted in one inoculated and one noninoculated (control) plot. Six weeks after seed germination, seedlings in all plots were thinned to a density of 270 per m^2. Seedlings were watered as needed during the growing season. In late June, all plots were fertilized with 56 kg N/ha as $NH_4 NO_3$.

In February 1974, roots of all seedlings were cut vertically between rows and undercut at a depth of 20 cm with a flat shovel. Seedlings were lifted by hand and graded. Loblolly and shortleaf pine seedlings were graded to heights of 20 and 15 cm ($\pm$ 10%) and root collar diameters of 0.3 and 0.4 cm, respectively, for both ectomycorrhizal treatments. Seedlings with less than 70 to 80 percent of their feeder roots in an ectomycorrhizal condition were discarded. Control seedlings had the majority of their ectomycorrhizae formed by *T. terrestris* and seedlings from soil infested with *P. tinctorius* had at least 90 percent of their total ectomycorrhizae formed by *Pisolithus*; the remainder were formed by *Thelephora*. Ectomycorrhizal assessments were done visually (Marx and Bryan, 1975). Seedlings were stored in moist peat moss in plastic-lined, kraft paper seedling bags at 5°C for 1 month prior to planting.

Study Installation: The planting site was located on a mountain top approximately 32 km north of London, Kentucky. The entire mountain top was strip-mined in 1971 leaving a spoil of approximately 8 ha. The spoil was planted in the fall of 1972 and again in 1973 with loblolly pine seedlings produced in a conventional tree nursery in Kentucky. Less than 10 percent of these seedlings survived; those living were severely stunted. The survivors were removed from the area prior to installation of this experiment.

Five blocks, each with eight 5×5 m plots, were installed on the spoil. A 3 m wide non-planted border separated each plot. One of eight treatments was randomly placed in each of the 8 plots per block. The treatments in the random block design were loblolly or shortleaf pine seedlings with either *Pisolithus* or *Thelephora* ectomycorrhizae planted with or without a fertilizer tablet. One tablet (21 gm, 20-10-5 formulation, Sierra Chemical Co., Newark, CA 94560) per seedling was placed in the closing hole approximately 8 cm deep and 8 cm from the seedling roots. Twenty-five seedlings were planted by hand in each plot in 5 rows of 5 seedlings each.

Spacing of seedlings between and within rows was approximately 1.25 m. Several spoil samples were removed to a depth of 0 to 15 cm from each plot, combined into one composite sample per plot and chemically analyzed.

Seedling Data: Two weeks after planting, height and root collar diameters were obtained from each seedling to confirm that seedlings of both pine species, regardless of initial ectomycorrhizal treatment, were of comparable size at planting. This was done to eliminate any possiblility of interaction between seedling size and field performance (Wakeley, 1954).

Measurements of survival, height and root collar diameters were obtained in November 1975, October 1976, and December 1977. Also, at each measurement period incidence of sporophores of *P. tinctorius* and *T. terrestris* were recorded and roots were removed from 3 random seedlings per plot and examined for specific ectomycorrhizae and hyphal strands of fungi. In 1976 and 1977, current-year needle samples were removed from 3 seedlings per plot and chemically analyzed for major and minor elements using routine procedures.

Survival and growth data after each growing season were integrated into indices of plot volume. Height × (root collar diameter)² was considered to be an indicator of seedling volume. Plot volume index (PVI) was determined by multiplying mean seedling volume by the number of surviving seedlings per plot (Marx et al., 1976). Analyses of variance were made on all data and differences among means were evaluated with Duncan's New Multiple Range Test (P=0.05).

Virginia Coal Spoil

Seedling Production: Loblolly pine seedlings (Virginia seed source) with *Pisolithus* (isolate 138) or *Thelephora* ectomycorrhizae outplanted on the Virginia coal spoil were grown under the same conditions used to produce seedlings for the Kentucky coal spoil. These seedlings were grown, however, from April 1973 to March 1974, at which time they were lifted. Unfortunately, while in the nursery a warm period occurred in January causing many seedlings to break dormancy and grow vigorously. Many were killed by subsequent frosts, reducing the number of seedlings available for outplanting. Because of this occurrence and resulting variables in seedling size the degree of ectomycorrhizal development was the main criteria for grading. Graded seedlings from the *Pisolithus* plot had a total ectomycorrhizal development of 85 percent of

which about 90 percent were formed by *Pisolithus*; the remainder was formed by *Thelephora*. Seedlings from the noninoculated, control plot had a total ectomycorrhizal development of about 80 percent, all of which were formed by naturally occurring *Thelephora*. Seedlings were packaged, as described earlier, shipped to Virginia, and planted within two days of their arrival.

Study Installation: The site is located in Buchanan County in southwest Virginia. The area was strip-mined several years previously and was selected for the study due to high soil temperature and unusually low pH. The area had been unsuccessfully seeded with a grass mixture earlier. Six paired plots were placed at random on level areas on the spoil. Each plot was 6.1×6.1 m and was separated from its companion plot by a 7 m wide nonplanted area. Seedlings with either *Pisolithus* or *Thelephora* ectomycorrhizae were planted in a randomly selected plot of each pair. Twenty-five seedlings were planted by hand in each plot in 5 rows of 5 seedlings each. Spacing of seedlings between and within rows was 1.5 m. Spoil samples were removed from a depth of 0 to 15 cm from three random plots and analyzed for pH, organic matter, and nitrate-N.

Seedling Data: One month after planting, height measurements were obtained to determine possible differences in seedling size caused by ectomycorrhizal treatment and lack of size-grading at the nursery.

Measurements of survival, height and root collar diameters were obtained in October 1974, December 1975, March 1977, and December 1977. Incidence of sporophores was recorded and an evaluation of seedling root systems was done at these times using procedures described earlier. Unfortunately, vandals uprooted and removed over 10 percent of the test seedlings just prior to obtaining 2nd year measurements in December 1975. Second year survival data were obtained by counting the holes as surviving trees. Survival data of subsequent years were based on existing trees. Because of the vandalism, the plot volume index parameter was not used to compare treatment effects in this study. All data were statistically analyzed using the paired t-test at P=0.05.

RESULTS

Kentucky Coal Spoil

Chemical analyses of spoil samples collected at planting revealed that, by random chance,

seedlings with *Thelephora* ectomycorrhizae were planted in plots that were significantly less acid, had less total Fe, but more available P and Ca than plots planted to *Pisolithus* seedlings. The pH of the spoil in the loblolly pine plots was 4.1 in the *Pisolithus* plots and 4.6 in the *Thelephora* plots; in the shortleaf pine plots, the reactions were pH 4.1 and 4.3, respectively. Phosphorus levels were 3 and 7 p/m in the *Pisolithus* and *Thelephora* plots, respectively, for loblolly pine and 2 and 6 p/m for shortleaf pine, respectively. Calcium varied from 467 p/m in the *Pisolithus* plots to 1241 p/m in the *Thelephora* plots of loblolly pine and 483 p/m to 691 p/m for shortleaf pine of *Pisolithus* and *Thelephora*. Total Fe in the corresponding loblolly and shortleaf pine plots was 35 and 34 p/m for *Pisolithus* and 46 and 49 p/m for *Thelephora* plots, respectively. Amounts of K (47 to 52 p/m), Mg (81 to 145 p/m), Mn (12 to 27 p/m), Zn (5 to 10 p/m), and Al (4 to 6 p/m) did not vary significantly. Statistical comparison of seedlings measurements obtained 2 weeks after planting revealed that all seedlings of a pine species, regardless of initial ectomycorrhizal treatment, had the same height and root collar diameters.

Survival and growth of loblolly pine were significantly influenced by ectomycorrhizae and the fertilizer tablets (Table 1). After the first growing season, there was no difference in survival or height growth of loblolly pine seedlings due to the treatment but seedlings with *Thelephora* ectomycorrhizae without the fertilizer tablet had smaller root collar diameters than other seedlings. However, differences became more apparent after these parameters were calculated to plot volume indices (PVI). After the first year, seedlings with *Pisolithus* ectomycorrhizae and the tablet had significantly larger PVI than seedlings with *Thelephora* ectomycorrhizae either with or without the fertilizer tablet. Growth differences due to *Pisolithus* ectomycorrhizae were even greater after the 2nd and 3rd growing seasons. The effect of the fertilizer tablet was highly significant after the 2nd growing season, but by the end of the 3rd year seedlings with *Pisolithus* ectomycorrhizae only were as large as seedlings with the tablet.

The fertilizer tablet increased the PVI of seedlings with *Thelephora* ectomycorrhizae after 2 and 3 years. The severe winter of 1976-77 caused a significant degree of mortality among seedlings with *Thelephora* ectomycorrhizae after the 3rd year. The spoil froze to a depth of approximately 50 cm, which was accompanied by persistent dry and cold winds for several weeks. By early

Table 1. Survival and Growth of Loblolly and Shortleaf Pine Seedlings with Pisolithus or Thelephora Ectomycorrhizae and With or Without a Fertilizer "Starter" Tablet After 1, 2, and 3 Years on a Coal Spoil in Kentucky

Year	Treatment	Percent Survival	Height (m)	Root Collar Dia (cm)	PVI* (cm³) × 10²
		---------- Loblolly pine ----------			
1	*Pisolithus* with tablet	89a	0.41a	1.03a	11a
	Pisolithus without tablet	84a	0.38a	0.91a	7ab
	Thelephora with tablet	80a	0.39a	0.83ab	5b
	Thelephora without tablet	90a	0.30a	0.55b	2b
2	*Pisolithus* with tablet	89a	1.07a	2.71a	196a
	Pisolithus without tablet	84a	0.95a	2.52a	128b
	Thelephora with tablet	80a	0.84a	1.83ab	57c
	Thelephora without tablet	87a	0.55b	1.26b	22d
3	*Pisolithus* with tablet	80a	1.45a	3.69a	348a
	Pisolithus without tablet	80a	1.45a	3.84a	351a
	Thelephora with tablet	68b	1.06b	2.53ab	102b
	Thelephora without tablet	75ab	0.83b	2.15b	68c
		---------- Shortleaf pine ----------			
1	*Pisolithus* with tablet	71a	0.30a	0.93a	5a
	Pisolithus without tablet	66a	0.23ab	0.65ab	2b
	Thelephora with tablet	71a	0.24ab	0.67ab	2b
	Thelephora without tablet	69a	0.19b	0.48b	1b
2	*Pisolithus* with tablet	69a	0.79a	2.37a	84a
	Pisolithus without tablet	66a	0.55ab	1.64ab	36b
	Thelephora with tablet	70a	0.62ab	1.78ab	53b
	Thelephora without tablet	63a	0.41b	1.15b	9c
3	*Pisolithus* with tablet	69a	1.26a	3.52a	237a
	Pisolithus without tablet	64a	1.06b	3.08b	136b
	Thelephora with tablet	65a	0.84bc	2.34c	114b
	Thelephora without tablet	61a	0.65c	1.82d	27c

* PVI (Plot Volume Index), cm³=(root collar diameter, cm)² × height, cm × number surviving seedlings per plot.

Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

March 1977, about 75 percent of needles of all seedlings in each treatment were brown. Seedlings with *Pisolithus* ectomycorrhizae, however, survived better, formed new needles, and initiated height-growth at least 6 weeks earlier than seedlings with *Thelephora* ectomycorrhizae. More seedlings with *Thelephora* ectomycorrhizae and the fertilizer tablet died than from any other treatment. By the end of the 3rd year, loblolly pine seedlings with *Pisolithus* ectomycorrhizae, independent of fertilizer tablets, had 12 percent better survival, were 53 percent taller, and had 61 percent larger root collar diameters than seedlings with *Thelephora* ectomycorrhizae. These increases resulted in an increase of more than 310 percent in PVI. Independent of ectomycorrhizae, the fertilizer tablets decreased survival by 5 percent, increased height by 10 per-

cent, and increased root collar diameter by 4 percent after the 3rd year. These parameters increased the PVI by only 7 percent.

Survival of shortleaf pine seedlings was not affected by treatment at any time during the period including the winter of 1976-77 (Table 1). On the basis of PVI, *Pisolithus* ectomycorrhizae and the fertilizer tablets improved seedling growth as early as the 1st year in comparison to seedlings with only *Thelephora* ectomycorrhizae. By the end of the 3rd year, seedlings with *Pisolithus* ectomycorrhizae and the fertilizer tablet still outperformed others. Seedlings with *Pisolithus* ectomycorrhizae only were as large as seedlings with *Thelephora* ectomycorrhizae and the fertilizer tablet. As a group, shortleaf pine seedlings with *Pisolithus* ectomycorrhizae had 6 percent better survival, 56 percent greater height

Table 2. Concentration of Elements* in Needles Sampled After 2 and 3 Years From Loblolly and Shortleaf Pine Seedlings Planted on a Coal Spoil in Kentucky as Affected** by Pisolithus or Thelephora Ectomycorrhizae Interacting with Fertilizer "Starter" Tablets. Each Value is the Mean of Combined Samples from Three Random Seedlings from Each of Five Plots per Treatment Each Year

Treatment	Sample Year	%			P/m		
		N	Ca	S	Fe	Mn	Al
		---------------- Loblolly pine ----------------					
Pisolithus with	2	1.19b	.214ab	.131b	60b	656b	622b
fertilizer tablet	3	1.12b	.257a	.112ab	—	912b	877b
Pisolithus without	2	1.67a	.152b	.146b	58b	826ab	543b
fertilizer tablet	3	1.38a	.220a	.106b	—	986b	822b
Thelephora with	2	0.96b	.247a	.156b	72ab	1139a	784ab
fertilizer tablet	3	1.04b	.291a	.128a	—	1224a	1059a
Thelephora without	2	1.16b	.258a	.218a	92a	1116a	755a
fertilizer tablet	3	1.13b	.262a	.126a	—	1180a	909ab
		---------------- Shortleaf pine ----------------					
Pisolithus with	2	1.43ab	.195a	.146b	97b	627a	829ab
fertilizer tablet	3	1.24a	.214a	.108b	—	790b	913b
Pisolithus without	2	1.70a	.166a	.149b	140ab	682a	741b
fertilizer tablet	3	1.29a	.192a	.120ab	—	800b	869b
Thelephora with	2	1.27b	.203a	.201ab	105b	860ab	977ab
fertilizer tablet	3	1.02b	.254a	.122ab	—	984a	1285a
Thelephora without	2	1.41ab	.179a	.242a	214a	1067a	1157a
fertilizer tablet	3	1.31a	.246a	.148a	—	1010a	1278a

*P, K, Mg, and Zn were not significantly affected by the treatments.

**Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

growth and 59 percent larger root collar diameters in comparison to seedlings with *Thelephora* ectomycorrhizae. This resulted in a 165 percent greater PVI by the end of the 3rd year. Independent of the ectomycorrhizae, the fertilizer tablets at the end of the 3rd year increased survival by 7 percent, height by 23 percent and root collar diameters by 20 percent. This resulted in a 115 percent increase in PVI. These data suggest that shortleaf pine was more nutrient demanding than loblolly pine on this site.

Pisolithus ectomycorrhizae were found to totally dominate the root systems of both pine species with or without the fertilizer tablet in all *Pisolithus* plots. Few nonmycorrhizal roots were detected on any of these seedlings. By the end of the 2nd year, yellow-gold hyphal strands of this fungus were evident to the unaided eye in the upper 10 cm of spoil as far as 1.5 m from the closest seedling. Sporophore production by *P. tinctorius* was also abundant. After the 3rd year, loblolly pine with and without the fertilizer tablet had a yearly production of 2.6 and 2.1 sporophores/m² of spoil surface, respectively; shortleaf pine had 2.1 and 1.8 sporophores/m² of

spoil surface, respectively. Sporophores varied in size from 2 to 18 cm in diameter; most had stalks from 7 to 12 cm in height.

Root systems of seedlings of both pine species with *Thelephora* ectomycorrhizae, with and without fertilizer tablets, had a low incidence of *Thelephora* ectomycorrhizae. Less than half of the new lateral roots (those extending from the original root system) produced by the end of the 3rd year had visually identifiable ectomycorrhizae. Many young, nonmycorrhizal feeder roots were dark in color and appeared to be nonfunctional. Only a trace (less than 1 percent) of *Pisolithus* ectomycorrhizae were observed on these seedlings. No *Pisolithus* sporophores were observed in these plots. It is not known at this time why basidiospores of *P. tinctorius* from the *Pisolithus* plots did not form more ectomycorrhizae on the *Thelephora* seedlings. Only 11 sporophores of *T. terrestris* were detected in all plots of seedlings with *Thelephora* ectomycorrhizae during the 3 years of this study.

During root evaluation it was observed that the fertilizer tablets were barely recognizable after the 2nd year. By the end of the 3rd year

Table 3. Survival and Growth of Loblolly Pine Seedlings with Pisolithus or Thelephora Ectomycorrhizae After 1, 2, 3, and 4 Years on a Coal Spoil in Virginia

End of Year	Treatment	Percent Survival	Height (m)	Root Collar Dia (cm)	Seedling Vol (cm³)*
1	*Pisolithus*	95a	0.27a	0.7a	0.02a
	Thelephora	86b	0.23a	0.6a	0.01a
2	*Pisolithus*	92a	1.01a	3.1a	10.1a
	Thelephora	79b	0.81b	2.2b	4.4a
3	*Pisolithus*	81a**	2.00a	5.6a	64.5a
	Thelephora	68b**	1.54b	4.0b	26.6b
4	*Pisolithus*	81a**	2.99a	7.6a	173.8a
	Thelephora	67b**	2.14b	5.3b	61.8b

* Seedling volume=(root collar diameter, cm)² × height, cm.

**Decrease in survival due in part to tree removal by vandals after second year.

Means within a given year and tree species, but between treatments with a common letter are not significantly different at P=0.05.

only remnants of the tablets were found. Roots and ectomycorrhizae growing within 1 cm of the tablets were occasionally discolored, but visually they appeared to be functional.

The different treatments had a significant effect on concentrations of N, Ca, S, Fe, Mn, and Al in seedling foliage (Table 2). Phosphorus (0.101 to 0.124%), K (0.5 to 0.62%), Mg (0.093 to 0.134%), and Zn (24.2 to 36.1 p/m) were not significantly affected by treatment. Loblolly pine seedlings with *Pisolithus* ectomycorrhizae planted without the fertilizer tablet had a significantly higher concentration of foliar N than seedlings of the other three loblolly pine treatments after the 2nd and 3rd growing seasons. Seedlings with *Thelephora* ectomycorrhizae generally had higher concentrations of S, Fe, Mn, and Al in foliage than seedlings with *Pisolithus* ectomycorrhizae regardless of the fertilizer treatment.

Shortleaf pine seedlings with *Thelephora* ectomycorrhizae planted with the fertilizer tablet had lower concentrations of foliar N after both years and more Fe after the 2nd year than seedlings in other treatments. Calcium content was not affected. Generally, seedlings with *Thelephora* ectomycorrhizae had higher concentrations of foliar S, Fe, Mn, and Al than seedlings with *Pisolithus* ectomycorrhizae regardless of the fertilizer treatment.

Virginia Coal Spoil

Analysis of height measurements obtained 1 month after planting showed that the loblolly pine seedlings with *Pisolithus* ectomycorrhizae (12.4 cm) were significantly taller (29 percent) than seedlings with *Thelephora* ectomycorrhizae (9.6 cm). Spoil analysis revealed a pH of 3.4, organic matter of 1.5 percent, and less than 5 p/m of nitrate-N.

Pisolithus ectomycorrhizae significantly decreased mortality of seedlings after the 1st year, and increased all parameters of seedling growth by the 2nd year (Table 3). Seedling volumes were 129, 143, and 181 percent greater for seedlings with *Pisolithus* ectomycorrhizae than those with *Thelephora* ectomycorrhizae at the end of years 2, 3, and 4 respectively. *Pisolithus* seedlings which were initially 3 cm or 29 percent taller than those with *Thelephora* ectomycorrhizae were 85 cm or 40 percent taller by the end of the 4th year.

Seedlings in this study were also damaged by the winter of 1976-77 and had identical needle symptoms as seedlings on the Kentucky spoil. However, unlike those on the Kentucky spoil where all seedlings showed the same amount of needle burn in early March, the degree of injury on seedlings on the Virginia spoil varied by treatment. Seedlings with *Thelephora* ectomycorrhizae incurred 25 percent needle burn, but only about 5 percent of the needles were burned on seedlings with *Pisolithus* ectomycorrhizae. The more severe needle burn on *Thelephora* seedlings which reduced growth during the following year probably accounted for some of the growth differences between these seedlings and those with *Pisolithus* ectomycor-

rhizae. Seedlings with *Pisolithus* ectomycorrhizae had darker green foliage, longer needle length and more dense foliage than seedlings with *Thelephora* ectomycorrhizae during the entire 4 years.

The results of root examination of seedlings in this test were similar to those on the Kentucky coal spoil. Hyphal strands and ectomycorrhizae of *Pisolithus* dominated roots of seedlings in the *Pisolithus* treatment, whereas only *Thelephora* ectomycorrhizae occurred erratically on seedlings in the *Thelephora* treatment. A few *Pisolithus* ectomycorrhizae were observed on the *Thelephora* seedlings after the 2nd season. Sporophore production by *Pisolithus* was prolific in the *Pisolithus* plots. During the fall of 1977, over 200 sporophores of *Pisolithus* were observed in the six *Pisolithus* plots (1.1 sporophore/m²) and 16 in the *Thelephora* plots. Sporophore size and shape were similar to those observed on the Kentucky spoil. *Thelephora* sporophores occurred occasionally in the *Thelephora* plots.

DISCUSSION

There is no doubt that *Pisolithus* ectomycorrhizae improved the survival and growth of loblolly and shortleaf pine seedlings on acid coal spoils. Seedlings with this ectomycorrhizal fungus had a root system that was physiologically capable of tolerating the adverse soil conditions and increasing absorption of essential nutrients from low concentrations. Due to a better physiological condition, these seedlings also exhibited fewer symptoms and recovered more rapidly from severe winter injury. Seedlings with *Thelephora* ectomycorrhizae not only grew more slowly, but they also accumulated higher levels of S, Fe, Al, and Mn in their needles than did seedlings with *Pisolithus* ectomycorrhizae. The significance of accumulation of these elements in needle tissue is not understood at this time, but high concentrations may reflect a physiological condition related to poor overall growth.

The fertilizer tablet improved growth of both pine species on the Kentucky coal spoil for the 1st year, but this effect did not continue through the 3rd year for loblolly pine. In fact, loblolly pine seedlings with *Pisolithus* ectomycorrhizae without fertilizer were as large as those with the fertilizer after 3 years. The gradual decrease in the effects of the fertilizer on loblolly pine was also detected in the needle analysis. Generally, after 3 years needles of seedlings with the fer-

tilizer tablet were not as green as those without the tablet. Nitrogen concentrations in needles were correlated with needle color, i.e., slightly chlorotic needles had less N than those with a darker green color. Apparently the soluble nutrients in the tablets were readily available and stimulated growth for only the 1st or 2nd growing season. Afterwards, nitrogen and perhaps other nutrients became limiting and the seedlings developed symptoms of N-deficiency. The N-deficiency was more striking in seedlings with *Thelephora* ectomycorrhizae than in those with *Pisolithus* ectomycorrhizae.

The influence of the fertilizer tablet on growth of shortleaf pine lasted the entire 3 years of this test. Regardless of the ectomycorrhizal condition, seedlings with the tablet were larger than those without the tablet. However, some needle chlorosis was observed on seedlings planted with the tablet at the end of the 3rd year. This was correlated with a decrease in foliar N, especially in seedlings with *Thelephora* ectomycorrhizae. The deeper rooting habit of shortleaf pine, as compared to loblolly pine, may account for the more prolonged response of shortleaf pine to the fertilizer tablet. Nutrients, leached from the tablet moved downward through the spoil, and were utilized more effectively by the deep roots of shortleaf pine. Perhaps, more slowly available forms of nutrients, such as those in sewage sludge (Berry, In press) would be more advantageous for long term benefits to seedling growth on acid coal spoils.

It can be concluded that survival and growth of pine seedlings on acid coal spoils were significantly improved by ectomycorrhizae formed by specific fungi ecologically adapted to these spoils. In these studies, *Pisolithus tinctorius* spread rapidly to newly formed roots, grew extensively throughout the spoil, and reproduced in abundance. In contrast to this is *Thelephora terrestris* which did not spread rapidly to newly formed roots or reproduce well on the spoil. *Thelephora* obviously was not well adapted to conditions inherent on acid coal spoils because seedlings that initially had abundant *Thelephora* ectomycorrhizae at planting eventually suffered from a low incidence of functional ectomycorrhizae shortly after planting. Other ectomycorrhizal fungi, such as *Scleroderma aurantium*, *Amanita rubescens* and others (Schramm, 1966; Medve et al., 1977), have been reported to occur frequently on acid coal spoils. These fungi should be tested under field conditions, such as reported herein, to ascertain their adaptability to coal spoils and their effect on seedling growth.

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DEELY

Inoculation of Nursery Seedbeds With Pisolithus Tinctorius Spores Mixed With Hydromulch Increases Ectomycorrhizae and Growth of Loblolly Pines

Donald H. Marx, John G. Mexal, and William G. Morris

ABSTRACT. Different methods of introducing basidio-spores (4/5 oz. spores/100 linear ft. of nursery bed) of Pisolithus tinctorius into fumigated soil at Weyerhaeuser's nursery in Oklahoma were tested to determine their effectiveness in forming ectomycorrhizae on loblolly pine seedlings. Two of five methods proved significantly effective. Nearly three-fourths of seedlings treated with spores mixed in hydromulch and applied after sowing formed Pisolithus ectomycorrhizae. The result was 25 percent larger seedlings and 15 percent fewer culls. In plots where spores were dusted onto the soil at sowing, one-third of the seedlings formed Pisolithus ectomycorrhizae, resulting in 12 percent larger seedlings and 13 percent fewer culls.

Considerable research has been conducted recently on the use of pure cultures of fungi to increase ectomycorrhizal development and growth of forest tree seedlings. In greenhouse studies in Australia, Theodorou (1971) and Theodorou and Bowen (1973) found that coating seeds of *Pinus radiata* with basidiospores of *Rhizopogon luteolus* is an easy and effective method to introduce ectomycorrhizal fungi into potting soil. In Australia, Lamb and Richards (1947a, b, c) used basidiospores of *R. roseolus*, *Suillus granulatus*, and *Pisolithus tinctorius* to increase ectomycorrhizal development and growth of *P. radiata* seedlings in natural soils deficient in fungal symbionts.

In this country, *P. tinctorius* has been successfully introduced into soils of nurseries in Florida and North Carolina (Marx and others 1976), Oklahoma (Marx and others 1978), Virginia (Marx and Artman 1978), and other southern states (unpublished data). In most cases the ectomycorrhizae formed with *P. tinctorius* significantly increased seedling growth of several species of pine. When pine seedlings with abundant *Pisolithus* ectomycorrhizae were planted on southern reclamation (Marx 1977) and routine reforestation sites (Marx *et al.* 1977), they survived and grew better than routine nursery seedlings having naturally occurring ectomycorrhizae. In the nursery tests, vegetative mycelium grown in pure culture in the laboratory in vermiculite-peat moss-nutrient medium was found to be consistently better than basidiospores for synthesizing ectomycorrhizae and stimulating

seedling growth. The effectiveness of basidiospore inoculum was decreased in those nurseries where there was intense competition from other ectomycorrhizal fungi. With minimum competition, such as was encountered at the Oklahoma nursery, the basidiospores were very effective in forming ectomycorrhizae and stimulating seedling growth (Marx *et al.* 1978). On the basis of these results, further research on the use of basidiospores appeared justified. We therefore tested different methods of introducing basidiospores of *P. tinctorius* into fumigated soil at the Oklahoma nursery to ascertain their effect on ectomycorrhizal development and growth of loblolly pine (*Pinus taeda* L.) seedlings.

MATERIALS AND METHODS

Dry basidiospores of *P. tinctorius* were extracted (Marx 1976) from mature basidiocarps collected in September 1975 under loblolly pine growing on a kaolin spoil near Macon, Georgia. The dry basidiospores were stored in amber bottles at 5°C. The amount of basidiospores used for all treatments in this test was approximately 4/5 oz./100 linear ft. of nursery bed. The experiment was installed at Weyerhaeuser's Fort Towson, Oklahoma nursery in a section where loblolly pine seedlings grew the previous year. In early spring of 1976, the soil was fertilized with 750 lb./acre of NH_4NO_3, 500 lb./acre of 12-12-12, 750 lb./acre of dolomitic lime, and 600 lb./acre of 10-20-20. All fertilizers were broadcast and disked into the soil. The nursery section also was sprayed with Captan® (12 lb./acre) and Terrachlor® (1 pt./acre) prior to sowing. The soil was fumigated in March 1976 with methyl bromide (MBC-2) applied under clear plastic at the rate of 318 lb./acre. The plastic was removed after 3 to 5 days, and the beds were shaped. Each of the five nursery units used in this test had nine 4- by 600-ft. beds. A 100-ft.-long test plot was laid out on both ends of beds 4, 6, and 8 in each unit to provide a total of 30 plots. Each nursery unit of six test plots was a

replicate block; each block contained one plot of each of six treatments.

The following six treatments were randomly installed in the six plots of each of the five nursery units.

Treatment 1.—Basidiospores mixed in hydromulch after sowing.

Loblolly pine seeds were sown prior to installing the treatment on April 7, 1976. Approximately 4 oz. of basidiospores were mixed thoroughly in 1.5 gallons of water with 30 drops of wetting agent (Tween 20[10]) in a 2-gallon plastic bottle. The basidiospore suspension was poured into a conventional Bowie[10] hydromulcher containing 275 gallons of water, 83 lb. of hydromulch (Silvafiber[10], Weyerhaeuser Company), and 13 quarts of sticker (Petroset SB[10], Phillips 66). This volume, only one-third of a load for the hydromulcher, was spread evenly over the 100-ft. plots in each of the five units within 30 minutes of adding the spores to the hydromulcher.

Treatment 2.—Basidiospores dusted onto soil after sowing.

Plots were seeded and irrigated to moisten the soil just prior to treatment application on April 8, 1976. Approximately 4 oz. of basidiospores were placed in a small, hand-operated pesticide duster (Pestmaster Garden Duster[10], D. B. Smith & Co., Utica, New York). Spores were dusted evenly on the moist soil of the five plots during a wind-free period. The plots were immediately irrigated and covered with hydromulch.

Treatment 3.—Basidiospores injected into soil before sowing.

Four ounces of spores were placed in a 3-gallon, pressurized paint sprayer and injected 4 inches deep into the soil through five shanks spaced 10 inches apart on a tractor-drawn rig; N_2 gas was used as the carrier. The five plots were seeded and hydromulched after the spores were injected on April 8, 1976.

Treatment 4.—Basidiospores dusted onto seedlings 6 weeks after sowing.

Plots were seeded and hydromulched on April 8, 1976. Six weeks later, on May 12, plots were irrigated to moisten the seedlings and the soil. Basidiospores were then dusted onto seedlings and the soil at the same rate and method used in Treatment 2. Immediately after dusting, the plots were irrigated to wash the spores into the root zone.

Treatment 5.—Basidiospores drenched onto seedlings 6 weeks after sowing.

In this treatment, plots were seeded and hydromulched on April 8, 1976. On May 13, basidiospores were placed in the hydromulcher containing water and sticker only (hydromulch fiber was omitted) and then drenched onto seedlings at the same rate and method used in Treatment 1.

Treatment 6.—Controls without basidiospores.

Control plots were treated as other plots, but spores were not added to the soil.

Loblolly pine seed used in this test were collected from a Mississippi-Alabama source in 1975 and treated with Latex and Arasan[10] (42-S). The rate of hydromulch was 1200 lb./acre with 2.4 percent Petroset SB[10] by volume.

During the growing season, all plots were fertilized uniformly with $(NH_4)_2SO_4$ and KCl according to the following schedule: June, 25 lb. N/acre; July, 30 lb. N/acre; August, 30 lb. N/acre; in the fall the plots were fertilized with 50 lb. K/acre to promote cold hardening of seedlings.

Seedlings were lifted and evaluated during the week of January 25, 1977. Prior to lifting, all plots were undercut with a root-pruning bar at a depth of 8 inches and laterally pruned between seedling rows. All seedlings were removed by hand from five 2- by 4-ft. subplots spaced 1, 25, 50, 75, and 99 ft. from one end of each 100-ft. test plot. These seedlings were counted and graded. Seedlings less than 6 inches tall, with stems less than 1/8 inch in stem diameter at the root collar, with forked tops, or without dormant buds were counted but discarded as culls. Ten plantable seedlings from each subplot were chosen at random for measurement of stem height, stem diameter at root collar, and fresh weights of shoots and roots. The degree of ectomycorrhizal development on each seedling was estimated visually (Marx *et al.* 1976). Data from subplots were totaled and averaged to represent the mean for the plot.

The seedlings in the 90 linear feet of nursery bed remaining per plot were machine-lifted and graded by Weyerhaeuser personnel on a conveyor line. On the conveyor line, seedlings less than 6 inches tall or taller than 14 inches and with stems less than 1/8 inch in diameter were counted as culls and discarded. Over 38,000 seedlings were removed from subplots by hand for detailed seedling evaluation, and over 310,000 seedlings were lifted by machine and graded on the conveyor line by Weyerhaeuser personnel. The data were subjected to analyses of variance. Differences among treatment means were separated with Duncan's Multiple Range Test.

Mixing basidiospores in hydromulch at sowing (Treatment 1) was the most effective method used in this study (Table 1). Nearly three-fourths of the seedlings had *Pisolithus* ectomycorrhizae, and approximately one-fourth of the total amount of ectomycorrhizae on seedlings was formed by *Pisoli-*

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Table 1. Seedling and plot data from loblolly pine grown in nursery beds infested by different methods with basidiospores of the ectomycorrhizal fungus *Pisolithus tinctorius* (Pt)[1]

Basidiospore inoculation method	Seedling data[2]					Plot data[3]	
			Fresh wt of 1,000 seedlings	Pt ectomycorrhizae			
	Height	Stem diam.		All ectomycor.	Seedlings	Total seedlings	Plantable seedlings
	Inches		*Lb.*	*Percent*		*Number*	*Percent*
Mixed in hydromulch after sowing	8.0 a	0.20 a	34 8 a	28 a	72 a	54,350 a	80.4 a
Dusted onto soil after sowing	7.6 b	0.18 b	30.8 b	10 b	36 b	50,575 b	78.7 a
Injected into soil before sowing	6.9 c	0.17 b	29.1 bc	6 c	24 c	50,405 b	74 3 ab
Dusted onto seedlings 6 weeks after sowing	7 4 ab	0.18 b	29 1 bc	6 c	26 c	52,015 ab	70.5 b
Drenched onto seedlings 6 weeks after sowing	7.2 bc	0.18 b	28.9 bc	4 c	22 c	50,650 b	69.3 b
Control (no basidiospores added)	7.0 c	0.17 b	27.5 c	0.2 d	0.01 d	52,770 ab	69 7 b

[1] Column means followed by a common letter are not significantly different (P = 0.05).
[2] Means obtained from 10 randomly selected seedlings lifted by hand from each of five 8-sq.-ft. subplots located in each of 5 blocks per treatment.
[3] Seedlings machine-lifted from five 90-linear-ft. plots per treatment (those remaining after seedlings removed from the five subplots); counted and graded by Weyerhaeuser personnel on conveyor line.

thus. These *Pisolithus* ectomycorrhizae induced a 25-percent increase in fresh weight of seedlings and increased the number of plantable seedlings by 15 percent over the controls. The next most effective treatment for forming *Pisolithus* ectomycorrhizae was dusting basidiospores onto soil at sowing (Treatment 2). Approximately one-third of the seedlings had *Pisolithus* ectomycorrhizae, and these represented 10 percent of all the ectomycorrhizae. This *Pisolithus* development, however, increased fresh weight of seedlings by 12 percent and the number of plantable seedlings by 13 percent over the controls. The remaining three treatments did not significantly affect seedling size or number of plantable seedlings. Very few seedlings in these latter treatments had *Pisolithus* ectomycorrhizae.

CONCLUSION

The mixing of basidiospores of *P. tinctorius* with hydromulch and applying them with the hydromulcher after seeding is a simple, rapid, and practical method to infest nursery soil with *P. tinctorius*. This method does not require any change in normal nursery operations, assuming that these operations include hydromulching. Initially, there was concern that the basidiospores would not leach from the wood fiber into the root zone, especially after the addition of the sticker to the wood fiber. This concern, as well as possible toxicity of the sticker to the spores, was unfounded. In a separate test (unpublished) in Athens, spores were mixed with various amounts of hydro-

mulch and sticker for different periods of time prior to mulching soil. It was found that hydromulch and sticker had no significant effect on the function of basidiospores as inoculum for *Pisolithus* ectomycorrhizal development.

Use of basidiospores of *P. tinctorius* instead of vegetative inoculum to infest soil in nursery operations has the following disadvantages and advantages.

Disadvantages:

1. Special efforts must be made to collect and store spores. There are also good and poor years for basidiocarp production in the field. Generally, every other year is a good year, with adequate and timely rainfall. As of this date, basidiospores are not commercially available.

2. Basidiospore collections are often contaminated with other microorganisms (yeast, bacteria, and fungi) and insects which may or may not affect health of nursery seedlings or viability of basidiospores.

3. Viability of basidiospores cannot be easily determined since they cannot be germinated consistently. The only reliable means of determining viability of basidiospores is to form ectomycorrhizae with them; this takes a minimum of three months usually.

4. Basidiospores are not as effective as vegetative inoculum in forming ectomycorrhizae on pine seedlings in nurseries. Spores take approximately 12 to 15 weeks to form detectable ectomycorrhizae, whereas vegetative inoculum takes less than 4 to 6 weeks (Marx *et al.* 1976).

5. In nurseries where recolonization of fumigated soil by naturally occurring ectomycorrhizal fungi is highly efficient, the effectiveness of basidiospores is reduced. This is not the case with vegetative inoculum following effective soil fumigation.

6. Basidiospores rarely form ectomycorrhizae on all seedlings in inoculated soil, and, with few exceptions, the overall development of ectomycorrhizae on seedlings at the end of the growing season is less than that formed by vegetative inoculum. This point is significant since increased survival and growth of seedlings in the field is correlated with the greatest amount of *Pisolithus* ectomycorrhizae (Marx *et al.* 1977).

Advantages:

1. In the South, basidiocarps can be found under pine and oak trees in great abundance on adverse sites, such as kaolin and coal spoils, especially in late summer and early fall. Several pounds of spores can be collected in only a few hours if basidiocarps are abundant. *Pisolithus* basidiocarps are easily identified since *Pisolithus* is the only fungus that produces its spores in small compartments inside the basidiocarp.

2. The spores can be extracted from the mature basidiocarps simply and economically (Marx and Bryan 1975; Marx 1976).

3. Dry spores can be stored effectively under refrigeration for at least 34 months (Marx 1976) and perhaps longer. Storage is essential since spores are normally collected in the summer or early fall and, in most instances, would not be used until the following spring.

4. Transporting basidiospores is considerably easier and more practical than transporting bulky vegetative inoculum.

5. The use of spores as inoculum for ectomycorrhizal development does not require an extended growth phase under aseptic laboratory conditions as does vegetative inoculum of *P. tinctorius* (Marx and Bryan 1975).

6. Results of this study indicate that no special equipment is needed to infest soil with basidiospores if a hydromulcher is available at the nursery.[1]

[1] This paper reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate state or federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—

if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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DEELY

ROLE OF MYCORRHIZAE IN FORESTATION OF SURFACE MINES[1]

Donald H. Marx[2]

Abstract.--A brief introduction to ecto- and endomycor-
rhizae and their importance to plants is presented. Recent
findings confirm the significance of ectomycorrhizae,
particularly those formed by *Pisolithus tinctorius* in
nurseries, to survival and growth of pine seedlings on strip-
mined lands. Commercial inoculum of this fungus may be avail-
able in 1981. Recent research shows that endomycorrhizal
fungi affect growth of grasses and certain hardwood trees on
coal spoils.

INTRODUCTION

Mycorrhiza (fungus-root) is a symbiotic
association between the fine feeder roots of
green plants and highly specialized root-
inhabiting fungi. Each partner in the mycor-
rhizal association depends on the other to one
degree or another for existence. In natural
forest soils, which are low in fertility in
comparison to artificially fertilized agri-
cultural soils, mycorrhizal associations are
essential to forest plants. These associations
increase (1) nutrient and water absorption and,
thus, nutrient cycling, (2) feeder root health
and longevity, and (3) tolerance to drought,
high soil temperatures, soil toxins (inorganic
and organic), and extremes of soil pH. Most
trees must have mycorrhizae to survive; other
plants may only need them for maximum growth
in natural soils.

With the exception of aquatics, some
halophytes and a few other plants, the
majority of flowering plants in the world
form either ecto- or endomycorrhizae in
natural soils (Mollock and others 1980).

Ectomycorrhizae occur naturally on many
important forest tree species around the world.
All members of the gymnosperm family *Pinaceae*
(pine, spruce, fir, larch, hemlock, etc.) as
well as certain angiosperms (willow, poplar,
aspen, hickory, pecan, oak, birch, beech,
eucalypt, etc.) normally form ectomycorrhizae.
Some of these trees can be either ectomycor-
rhizal or endomycorrhizal, depending on soil
conditions. Ectomycorrhizal fungal infection
is initiated from spores or hyphae (propagules)
of the fungal symbionts inhabiting the rhizo-
sphere of the feeder roots. Propagules are
stimulated by root exudates and grow over the
feeder root surface and form a fungus mantle.
Then hyphae develop around root cortical cells
and form what is called the Hartig net. The
Hartig net is the main distinguishing feature
of ectomycorrhizae. Ectomycorrhizal roots may
be unforked, bifurcate, multiforked (corralloid),
nodular, or in other shapes. The color of an
ectomycorrhiza is usually determined by the
color of the hyphae of the fungal symbiont and
may be brown, black, white, red, yellow, or
blends of these colors. Individual hypha,
strands of hyphae, or rhizomorphs may radiate
from the fungus mantles into the soil and to
the base of the fruit bodies of the fungi.

Most fungi which form ectomycorrhizae with
forest trees are Basidiomycetes that produce
mushrooms or puffballs (fruit bodies). Certain
Ascomycetes such as truffles also form ectomy-
corrhizae. One genus of mushroom-producing
fungi, *Cortinarius*, is composed of over 2000
species distributed throughout the forests of
the world; all species form ectomycorrhizae.
If other genera are considered, over 5000 ecto-
mycorrhizal fungus species probably exist--
including 2100 in North America. The fruit
bodies of these fungi produce billions of spores
that are widely disseminated by wind and water.
Most ectomycorrhizal fungi depend on their
hosts for simple carbohydrates, amino acids,
vitamins, etc. necessary to complete their
life cycles. Ectomycorrhizal development,

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therefore, is a prerequisite for fruit body production by these fungi. Not all fungi which form mushrooms and puffballs are ectomycorrhizal; many are litter decomposers and a few are pathogenic on trees.

Endomycorrhizae are formed on most economically important agronomic and forage crops, woody perennials, ornamentals, fruit and most nut trees, as well as maple, elm, green ash, sweetgum, sycamore, black walnut, and other important forest trees. Alder can form both endo- and ectomycorrhizae as well as symbiotic N-fixing nodules--three different symbiotic root associations at one time. Endomycorrhizal fungi form a loose network of hyphae on feeder root surfaces and do not develop the dense fungus mantle of ectomycorrhizae. These fungi often form large, conspicuous, thick-walled spores both on the root surfaces and in the rhizosphere, and sometimes in feeder root tissues. Hyphae of endomycorrhizal fungi penetrate the cell walls and progress into the cortical cells of the root. These infective hyphae may develop specialized absorbing or nutrient-exchanging structures (haustoria) called arbuscules in the cytoplasm of the cortical cells. Thin-walled, spherical to ovate vesicles may also be produced in cortical cells by these fungi. The term "vesicular-arbuscular" mycorrhizae has been coined to denote this type of endomycorrhizae. As in ectomycorrhizae, endomycorrhizal fungus infection rarely occurs in meristematic or vascular tissues. Endomycorrhizal infection, however, does not cause major morphological changes in roots. Endomycorrhizae, therefore, cannot be detected with the unaided eye; they must be assessed microscopically.

The fungi which form endomycorrhizae with trees are mainly Phycomycetes. They do not produce large, above-ground fruit bodies or wind-disseminated spores as do most ectomycorrhizal fungi, but some of them produce large spores on or in roots. Some species also produce large sporocarps (5 to 10 mm diameter) containing many spores on roots. These fungi spread through the soil by growing from feeder root to feeder root; they are also disseminated by moving water, soil, insects, or animals. They are so widespread that it is nearly impossible to find natural soils anywhere in the world that do not contain them. Spores of these fungi are able to survive in soil for many years without a plant host. Based on the limited research done on endomycorrhizal fungi, most species appear to have very broad host ranges. For example, *Glomus mosseae* is known to form endomycorrhizae with grasses, cotton, corn, pepper, tomatoe, peas, soybean, and sorghum, as well as sycamore, black walnut, sweetgum, citrus, peach, black locust, green ash, black cherry, boxelder, sugar maple, and red maple. It undoubtedly can infect numerous other plant

species as well. Since endomycorrhizal fungi cannot be grown routinely in the laboratory, production of inoculum is difficult. Usually a fast growing plant, such as sorghum, is used to culture these fungi. Roots and soil from these plants containing the introduced fungus are harvested and used as inoculum.

Many factors affect mycorrhizal development. Some factors affect the tree and others affect the fungal symbionts. Generally, any soil or above-ground condition which influences root growth also influences mycorrhizal development. A susceptible feeder root must be formed by the tree before mycorrhizal infection can occur. The main factors influencing susceptibility of tree roots to mycorrhizal infection appear to be photosynthetic potential and soil fertility. High light intensity and low to moderate soil fertility enhance mycorrhizal development; the other extremes of these conditions (light intensity below 20 percent of full sunlight and excessively high soil fertility) reduce, or may even prevent, mycorrhizal development. Light intensity and fertility appear to influence either the biochemical status of feeder roots, such as controlling levels of simple sugars, or the synthesis of new feeder roots, both of which are prerequisites to symbiotic infection. Roots growing rapidly because of very high soil fertility contain few simple sugars and they are not highly susceptible to ectomycorrhizal fungus infection (Marx and others 1977).

The factors that affect the fungal symbionts directly are those which regulate survival of the fungus in the soil or its growth on roots. Extremes of soil temperature, pH, moisture, etc., and the presence of antagonistic soil microorganisms can affect the survival of symbionts and thereby influence the mycorrhizal potential of the soil. Mycorrhizal fungi cannot grow and reproduce in soil unless they are in symbiotic association with plant roots. These fungi are capable, however, of surviving in a dormant condition for several years without a plant host.

There are several excellent reviews available on ectomycorrhizae (Malloch and others 1980, Marx and Krupa 1978, Marks and Kozlowski 1973) and endomycorrhizae (Malloch and others 1980, Sanders and others 1978, Hayman 1978).

Mycorrhizae and forestation of undisturbed soils

Failures in artificial regeneration programs have shown that many forest trees will not survive long after transplanting if the seedlings do not have an adequate complement of mycorrhizae from the nursery. Most reports deal with trees such as pines, spruces, oaks, and eucalypts and their establishment in areas of the world void of naturally occurring ecto-

mycorrhizal fungi. Mikola (1969) and, more recently, Marx (1980) discussed the need for a parallel introduction of ectomycorrhizal fungi into these areas. This introduction is especially critical in the successful establishment of exotic pine plantations in the tropics. These various reports have conclusively shown that (1) ectomycorrhizae formed by any fungus on roots of seedlings is better for the seedling than no ectomycorrhizae at all, and (2) ectomycorrhizae formed by certain species of fungi are more beneficial to seedlings on certain sites than ectomycorrhizae formed by other fungi. The value of specific ectomycorrhizae, such as that formed by *Pisolithus tinctorius*, to the improved survival and rapid early growth of pine seedlings has been demonstrated on various reforestation sites in the Southern United States (Marx 1980). Little work has been reported on the significance of endomycorrhizae to the artificial regeneration of hardwoods. It is known, however, that endomycorrhizae improve the quality of hardwood seedlings in the nursery (Kormanik and others 1977) which should also improve their field performance.

Ectomycorrhizae and forestation of surface mines

Marx (1975, 1976, 1977a) has reviewed the significance of specific ectomycorrhizae to survival and growth of pines on various spoils left after surface mining for coal and kaolin. Only a few key reports are mentioned here.

Schramm (1966) published a comprehensive report on plant colonization of anthracite wastes in Pennsylvania. He found that early ectomycorrhizal development was essential for the establishment of *Betula*, *Pinus*, *Populus*, and *Quercus* spp. seedlings which developed from seed on the wastes. Fruit bodies of the major fungi that developed near the surviving seedlings were the mushroom-forming fungi *Inocybe lacera*, *Amanita muscaria*, and *Thelephora terrestris*, and the puffball-producing fungi *Scleroderma aurantium* and *P. tinctorius*. The latter fungus appeared to be dominant and Schramm was able to trace its unique gold-yellow hyphal strands from similarly colored ectomycorrhizae to the base of the puffball. He associated this specific fungus with the most vigorously growing seedlings. Since Schramm's original work, numerous other reports on the occurrence of *P. tinctorius* on coal spoils and other adverse sites have been published.

Based on these observations and the conclusion that the natural occurrences of *P. tinctorius* ectomycorrhizae on seedlings growing on adverse sites were instrumental in their establishment and growth, we developed techniques to "tailor" bareroot and containerized seedlings in nurseries with *P. tinctorius*

ectomycorrhizae. Briefly, the techniques involve growing mycelium of *P. tinctorius* in pure culture in the laboratory in vermiculite-peat moss-nutrient substrate. After 2 to 3 months growth, the substrate is leached with water, broadcast onto fumigated nursery soil, and mixed thoroughly into the soil. The bed is then seeded. By the end of the growing season, abundant *P. tinctorius* ectomycorrhizae are developed on seedling roots. The seedlings with *P. tinctorius* ectomycorrhizae also have various quantities of wild-type ectomycorrhizae formed by naturally occurring fungi whose spores are wind disseminated into the fumigated nursery soil. The most prevalent wild-type fungus encountered is *Thelephora terrestris*. *T. terrestris* ectomycorrhizae are the most commonly found ectomycorrhizae on seedlings used for reforestation throughout the United States and in many other parts of the world. This fungus is ecologically adapted to good soil conditions such as those found in nurseries. It is highly beneficial to seedlings planted on routine sites, but is not well adapted to the harsh soil conditions normally encountered on strip-mined lands.

In earlier reviews (Marx 1975, 1976, 1977a), preliminary data showed the advantage of *P. tinctorius* ectomycorrhizae to survival and growth of pine seedlings on adverse sites as compared to seedlings with *T. terrestris* ectomycorrhizae. Very little work has been done on the significance of endomycorrhizae to hardwoods since the earlier reports. The following is the state of the art since the last review on the significance of mycorrhizae to forestation of surface-mined lands.

Ectomycorrhizae and forestation of strip-mined lands

Harris and Jurgensen (1977) observed that cuttings of willow and hybrid poplar grew poorly on copper tailings in Michigan. Ectomycorrhizae were not present even on cuttings inoculated with a forest soil extract supposedly containing ectomycorrhizal fungi. Copper tailings may have been toxic to indigenous or introduced inoculum of ectomycorrhizal fungi, or the physiological conditions of the rooted cuttings may have been inadequate for root infection to occur. A similar study was installed on iron tailings. Seedlings of both hardwood species grew well and formed abundant ectomycorrhizae from indigenous fungi and from those contained in the forest soil inoculum. Better seedling growth and development of ectomycorrhizae on the iron tailings could have been due to better soil fertility or fewer toxic chemicals.

Marx and Artman (1979) reported that bareroot loblolly pine seedlings with abundant *P. tinctorius* ectomycorrhizae had a 400 percent

greater plot volume after 3 years on an acid coal spoil (pH 4.1) in Kentucky than seedlings with *T. terrestris* ectomycorrhizae. On plots where fertilizer starter tablets were used volumes for *P. tinctorius* seedlings were nearly 250 percent greater. In the same test, shortleaf pine seedlings with *P. tinctorius* ectomycorrhizae were also over 400 percent larger without fertilizer tablets and over 100 percent larger with fertilizer tablets than seedlings with *T. terrestris* ectomycorrhizae. Seedlings with *P. tinctorius* ectomycorrhizae also contained more foliar N and less foliar S, Fe, Mn, and Al than seedlings with *T. terrestris* ectomycorrhizae. The fertilizer starter tablets stimulated seedling growth for the first and second growing seasons, but once the nutrients in the tablets were depleted growth increment slowed and N deficiency symptoms appeared on foliage. This deficiency was less striking on *P. tinctorius* seedlings than on seedlings with *T. terrestris*. In this same report, plot volumes for loblolly pine with *P. tinctorius* ectomycorrhizae were over 180 percent greater than those for seedlings with *T. terrestris* ectomycorrhizae after 4 years on an acid coal spoil (pH 3.4) in Virginia.

In two other experiments (C. R. Berry, unpublished data), container grown (root medium with 20 percent sewage sludge) loblolly, pitch, and loblolly x pitch pine hybrids with *P. tinctorius* or *T. terrestris* ectomycorrhizae were outplanted on acid coal spoils in Tennessee and Alabama. All loblolly pines were from a single parent and pitch pine were from two parent trees. After two and one-half growing seasons the results were striking (Table 1). On the Tennessee spoil, *P. tinctorius* ectomycorrhizae increased loblolly pine plot volumes by 585 percent and pitch pine plot volumes by 12 percent for one parent and 125 percent for the other. *P. tinctorius* ectomycorrhizae increased hybrid plot volumes by 120 to 575 percent. Overall, volumes on plots with *P. tinctorius* ectomycorrhizae were over 200 percent greater than plot volumes with *T. terrestris* ectomycorrhizae. On the Alabama spoil, loblolly pine plots with *P. tinctorius* ectomycorrhizae had nearly 300 percent greater volumes than those with *T. terrestris* ectomycorrhizae. The advantage in plot volumes of *P. tinctorius* over *T. terrestris* ectomycorrhizae was 150 and 240 percent for the pitch parents. The pitch pine parent that benefited relatively little on the Tennessee spoil (parent #78) was strongly stimulated by *P. tinctorius* ectomycorrhizae on the Alabama spoil. The reason for this is being investigated. The response of the hybrids to *P. tinctorius* ectomycorrhizae on the Alabama spoil was between 400 and 1400 percent. The overall increase in plot volume attributable to *P. tinctorius* on this spoil was over 350 per-

cent. Regardless of genotype, seedlings with *P. tinctorius* ectomycorrhizae had more N and less Ca, Mn, Zn, Cu, and Al in foliage than seedlings with *T. terrestris* ectomycorrhizae.

Many construction projects, such as dams, highways, and buildings, require extensive earth fill to meet design criteria. When insufficient soil fill is available on site it becomes necessary to "borrow" soil from another location. Generally, the resulting borrow pits are stripped excavations from which all the A and B soil horizons have been removed which present special problems in revegetation. Unlike coal or other surface mining spoils, borrow pits usually do not have toxic levels of heavy metals, extremes of soil acidity, or extremely high soil temperatures. Frequently, however, soil in borrow pits is highly compacted with poor internal drainage and have low levels of available plant nutrients and organic matter.

Two studies were installed on a borrow pit in Aiken, South Carolina, in which soil amelioration techniques and specific ectomycorrhizae were studied to determine their effects on the establishment of pines and grasses. The entire borrow pit was subsoiled to a depth of 0.9 m on 1.2 m centers in two directions. The first study (Berry and Marx 1980) measured effects of applying processed sewage sludge or fertilizer (560 kg/ha of 10-10-10) and dolomitic limestone (2240 kg/ha) with and without bark and/or bottom furnace ash on growth of loblolly pine and fescue grass. In the fall of 1975, sludge, bark, and ash were applied 1.3 cm deep (125 m^3/ha), and all plots were double disked. The following winter, loblolly pine seedlings with either *P. tinctorius* or *T. terrestris* ectomycorrhizae formed in a bareroot nursery were planted. By the end of the first growing season, root evaluation revealed that indigenous *P. tinctorius* on the borrow pit formed ectomycorrhizae on *T. terrestris* seedlings and, thus, negated the effects of "tailoring" seedlings with *P. tinctorius* in the nursery. However, sludge with or without the other organic amendments increased pine seedling volumes by 2800 percent and biomass of fescue grass by 500 percent. Soil amended with sewage sludge also contained more N, P, and organic matter, as well as a higher cation exchange capacity, than soil from non-sludge plots. Seedling foliage contained more N and less Ca in sludge plots. In the second test (Ruehle 1980), sludge or fertilizer + lime were similarly applied in the fall of 1975, but the container-grown loblolly pine seedlings with either *P. tinctorius*, *T. terrestris*, or no ectomycorrhizae (produced in a special growth room) were not planted until the fall of 1977. The interim between sludge application and seedling planting significantly decreased the indigenous levels of *P. tinctorius*

Table 1.--Survival and growth of loblolly and pitch pines and their hybrids with *Pisolithus tinctorius* ectomycorrhizae after 2-1/2 growing seasons on coal spoils in Tennessee and Alabama (C. R. Berry, unpublished data).

Pine line	Ectomycorrhizae at planting	Survival %	Height cm	Stem caliper cm	PVI[a] (x 10^2)
		Tennessee Spoil			
Loblolly 23	*P. tinctorius*	38	70	2.5	48
	Natural	48	40	1.3	7
Pitch 62	*P. tinctorius*	88	62	2.8	81
	Natural	93	47	2.0	36
Pitch 78	*P. tinctorius*	85	46	2.2	36
	Natural	94	42	1.8	32
62 x 11-9	*P. tinctorius*	88	89	3.3	169
	Natural	74	49	1.8	25
62 x 23	*P. tinctorius*	89	82	3.4	156
	Natural	76	55	2.0	41
62 x 11-20	*P. tinctorius*	90	84	3.2	156
	Natural	95	64	2.4	71
78 x 23	*P. tinctorius*	91	86	3.4	175
	Natural	81	54	2.1	48
58 x 11-20	*P. tinctorius*	90	87	3.1	162
	Natural	83	56	2.1	51
62 x 11-10	*P. tinctorius*	100	100	3.3	219
	Natural	85	66	2.2	78
Overall mean (TN)	*P. tinctorius*	85	79	3.0	133
	Natural	81	53	2.0	43
		Alabama Spoil			
Loblolly 23	*P. tinctorius*	79	76	2.9	126
	Natural	73	51	1.7	32
Pitch 62	*P. tinctorius*	41	57	3.0	41
	Natural	46	38	1.7	16
Pitch 78	*P. tinctorius*	69	52	2.6	48
	Natural	44	37	1.7	14
62 x 11-9	*P. tinctorius*	70	67	2.9	101
	Natural	59	46	1.6	19
62 x 23	*P. tinctorius*	63	69	3.2	106
	Natural	44	36	1.3	7
62 x 11-20	*P. tinctorius*	76	80	3.6	154
	Natural	68	50	1.7	30
Overall mean (AL)	*P. tinctorius*	66	67	3.0	96
	Natural	56	43	1.6	20

[a]Plot volume index (PVI) computed by (stem caliper, cm)^2 x height, cm x number of surviving seedlings per plot.

ectomycorrhizae in the soil, since the integrity of the ectomycorrhizal treatments on seedlings was reasonably maintained for the duration of the study. The effects of sludge and specific ectomycorrhizae were significant. After 2 years in sludge plots, seedlings with *P. tinctorius* ectomycorrhizae had 265 and 528 percent greater plot volumes than seedlings with *T. terrestris* or no ectomycorrhizae at planting. In the fertilizer + lime plots, the initially nonmycorrhizal seedlings had plot volumes 158 percent less

than the seedlings with either of the two ectomycorrhizal treatments. As a group, seedlings on sludge plots had 900 percent greater plot volumes than those on fertilizer plots. Differences in soil and foliar analyses and grass biomass were similar to those obtained in the other borrow pit study. After 2 years, the initially nonmycorrhizal seedlings had abundant ectomycorrhizae; *P. tinctorius* accounted for about 10 percent of these.

There appears to be little doubt that pine seedlings with *P. tinctorius* ectomycorrhizae survive and grow faster on adverse sites than routine nursery seedlings with naturally occurring ectomycorrhizae. These results indicate that seedlings for planting on adverse sites should be tailored in the nursery with *P. tinctorius* ectomycorrhizae. Until now, the only inoculum of *P. tinctorius* available was produced in small quantities on highly defined growth medium under rigidly controlled conditions in research laboratories. For commercial use, large volumes of highly functional inoculum of *P. tinctorius* are required. In 1976, the Institute for Mycorrhizal Research and Development, Athens, Georgia, joined with Abbott Laboratories[1] to devise means of producing vermiculite-based vegetative inoculum of this fungus in large fermentors. After 4 years of testing different formulations of inoculum in over 40 nurseries in 33 States and Canada, adequate procedures for producing functional inoculum have been accomplished. Preliminary results from our 1980 bareroot and container nursery tests in five Southern States are excellent. In 1980, a modified seeder was used for the first time to apply inoculum. This machine broadcasts inoculum, incorporates the inoculum into the root zone, levels the soil, and sows the pine seed in one pass over the nursery bed. This machine greatly simplifies application of vegetative inoculum. If the final results from the 1980 tests are as good as the preliminary results, inoculum will be available from Abbott Laboratories by early 1981.

The use of basidiospores of *P. tinctorius* as inoculum instead of the vermiculite-based inoculum also shows promise. There are certain advantages to the use of basidiospores: (1) no germ-free growth phase is required in fermentors, (2) they are easy to apply via hydromulch to nursery soil (Marx and others 1979), (3) after drying they store for years under refrigeration, and (4) they can be collected directly from adverse sites by the user. Unfortunately, basidiospores also have

certain disadvantages: (1) there are good and poor years for production of *P. tinctorius* fruit bodies in the field, (2) basidiospore collections are often contaminated with other microorganisms and insects which may or may not be harmful to nursery seedlings, (3) viability of basidiospores cannot be determined prior to use since successful germination procedures have not been developed, and (4) by far the most important disadvantage, basidiospores are usually not as effective in forming abundant *P. tinctorius* ectomycorrhizae early in the growing season on nursery seedlings as is properly produced vegetative inoculum. This latter point is very important since research results show that in order for seedlings to obtain maximum benefit from this fungus, at least half of all the ectomycorrhizae on the seedling roots must be formed by *P. tinctorius* (Ruehle and others[2]). For the past 3 years, adding basidiospores of *P. tinctorius* to encapsulated pine seed has also been studied; results look very promising. Since *P. tinctorius* forms ectomycorrhizae with numerous tree species (Marx 1979), namely, species of *Pinus*, *Quercus*, *Betula*, *Picea*, *Tsuga*, *Carya*, *Salix*, *Populus*, *Abies*, and *Eucalyptus*, its use could significantly improve reclamation efforts of a variety of strip-mined lands.

Endomycorrhizae and forestation of strip-mined lands

With the exception of the outplanted sycamore and sweetgum studies on kaolin spoils reported earlier (Marx 1977a), there are no published works on the effects of endomycorrhizae on growth of hardwoods or other plants tested directly on strip-mined lands. Since the last review, field observations and greenhouse studies have been reported on various plant species.

In Wyoming, Miller (1979) collected root samples from plants growing on a 2-year-old revegetated strip-mined spoil (previously segregated and stored topsoil replaced to a depth of 1 foot) and an adjacent undisturbed site. He found no endomycorrhizae on plant roots growing on the disturbed site but, with few exceptions, abundant endomycorrhizae were found on the diverse plant species growing on the adjacent undisturbed site. Inoculum of endomycorrhizal fungi was present in the disturbed soil, but was not infective. Miller speculated that the absence of infectivity by

[1]Dr. Donald S. Kenney, Abbott Laboratories, Agricultural Chemicals Research, 36 Oakwood Road, Long Grove, Illinois 60047.

[2]Ruehle, J. L., D. H. Marx, J. P. Barnett, and W. H. Pawuk. [In Press] Survival and growth of container-grown and bareroot shortleaf pine seedlings with *Pisolithus* and *Thelephora* ectomycorrhizae. Southern J. Appl. For:

the inoculum may be due to the presence of *Halogeton*, a herbaceous plant with suspected allelopathic capacity. He further speculated that disturbed areas are first colonized by pioneer plant species (*Halogeton* and other species) which are nonmycorrhizal and possibly allelopathic. Miller concluded that succession to mycorrhizal-dependent plant species should follow the elimination of *Halogeton*. Reeves and others (1979) found that 99 percent of the plant cover on a natural, undisturbed sage community in Colorado were endomycorrhizal, and that less than 1 percent of the plant cover on a disturbed area (subsoiled roadbed) were endomycorrhizal. In their opinion, the reestablishment and maintenance of the mycorrhizal fungus component is vital in producing stable plant ecosystems on disturbed areas. Using soils from these same sites in an endomycorrhizal bioassay, Moorman and Reeves (1979) found that the disturbed soil produced only 1/40 as much endomycorrhizal infection on corn as did the undisturbed soil. They concluded that the reduction of active inoculum in the disturbed soil was an important ecological factor in subsequent plant succession.

Daft and Hacskaylo (1976) found that most of the herbaceous plants sampled from anthracite and bituminous coal wastes in Pennsylvania were infected with endomycorrhizae; five naturally occurring plant species had both nodules and endomycorrhizae. In an inoculation test in a greenhouse, lucerne that was nodulated and infected with endomycorrhizae grew 700 percent better in limed (pH 6.4) anthracite waste than control lucerne plants. Regardless of inoculation treatment, however, lucerne plants died in limed (pH 5.8) or nonlimed (pH 2.7) bituminous wastes and unlimed (pH 4.1) anthracite wastes. In a later greenhouse study, Daft and Hacskaylo (1977) reported that red maple seedlings with endomycorrhizae were about 400 percent larger and contained more major elements in foliage after 70 days growth in limed (pH 6.2) anthracite wastes than seedlings without endomycorrhizae.

As discussed, the survival of endomycorrhizal inoculum on strip-mined lands appears important in plant succession. Various mechanical procedures carried out during strip mining can affect inoculum survival. Ponder (1979) assayed recently graded strip-mined coal spoil and found that plants grown in the spoil formed abundant endomycorrhizae in the greenhouse. He concluded that grading during reclamation could be an important means of dispersing endomycorrhizal inoculum in spoil. In unpublished reports[1], survival of endomycorrhizal fungi in piles of topsoil removed prior to strip mining of coal in Wyoming and North Dakota was found to be

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[1]Fourth North American Conference on Mycorrhizae, Colorada State Univ., Fort Collins, Colorado, June 24-28, 1979.

significantly reduced after 2- to 3-years-storage as compared to survival in nondisturbed topsoil or topsoil immediately replaced over the mine spoil.

CONCLUSION

It should be apparent from this discussion and earlier reviews (Marx 1975, 1976, 1977a) that mycorrhizae are not only essential to growth and development of trees in natural forest ecosystems, but are especially significant to their performance on strip-mined lands and other adverse sites. This biological fact has not been applied on any practical scale because procedures to manage the fungi have not been available to the land manager. The availability of commercial inoculum of *P. tinctorius* to tailor seedlings in the nursery prior to outplanting is the first step in closing this technology gap. We still have a long way to go, however, in the selection, inoculum production, manipulation, and management of other important ectomycorrhizal fungi, as well as all endomycorrhizal fungi. Research in these and related areas must continue so that sound scientific practices can be used in the forestation of adverse sites and in reforestation of routine forest sites.

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INOCULATION OF CONTAINERIZED LOBLOLLY PINE SEEDLINGS WITH BASIDIOSPORES OF *PISOLITHUS TINCTORIUS*

by John L. Ruehle[1]

ABSTRACT.—Loblolly pine seedlings growing in containers were inoculated with dry basidiospores of *Pisolithus tinctorius* 0, 2, 4, 6, or 8 weeks after transplanting. Eighteen weeks after transplanting, all seedlings were evaluated for ectomycorrhizal development. Seedlings inoculated at 0 or 2 weeks had more *Pisolithus* ectomycorrhizae than those inoculated later. The frequency of seedlings with *Pisolithus* ectomycorrhizae was greatest (98 percent) at 0 time. Naturally occurring *Thelephora terrestris* colonized all seedlings during this test and, through competition, was probably responsible for a lower rate of *Pisolithus* colonization on seedlings inoculated later than 2 weeks.

Keywords: Ectomycorrhizae, *Pinus taeda*, *Thelephora terrestris*.

Tree seedlings to be used in artificial regeneration have the potential for increasing forest productivity when they are inoculated with selective ectomycorrhizal fungi (Marx 1977). In nurseries, seedlings inoculated with symbionts ecologically adapted to different planting sites had improved survival and increased early growth when outplanted on routine reforestation sites (Marx and others 1977). Bare-root pine seedlings colonized with *Pisolithus tinctorius* have exhibited dramatic growth responses when outplanted on adverse sites such as coal spoils (Marx and Artman 1979) and severely eroded sites (Berry and Marx 1976). The potential value of inoculating containerized pine seedlings with ectomycorrhizae has been discussed (Marx and Barnett 1974), but the added benefit of using specific symbionts such as *P. tinctorius* for this purpose has received only limited testing.[2][3]

Basidiospores of *P. tinctorius* have been used successfully as inoculum in a bare-root nursery (Marx and others 1979) and in containers in a greenhouse (Marx and Barnett 1974; Ruehle and Marx 1977). The spores of *P. tinctorius* can be collected readily in large quantities from naturally occurring sporophores and can be stored for long periods. In most previous work involving inoculation, the spores were added to the growing medium before filling and seeding the containers, but this technique has not produced the degree of ectomycorrhizal development achieved with vegetative inoculum. However, since reasonably successful colonization of pine seedlings growing in natural soil in cans has been achieved by dusting basidiospores of *P. tinctorius* onto the soil surface (Lamb and Richards 1974), it was concluded that actively growing feeder roots might be essential to the successful use of spores as inoculum.

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[2]Ruehle, John L. Growth of containerized loblolly pine with specific ectomycorrhizae after 2 years on an amended borrow pit. [In process.]

[3]Ruehle, John L., Donald H. Marx, James P. Barnett, and William H. Pawuk. Survival and growth of container-grown and bare-root shortleaf pine seedlings with *Pisolithus tinctorius* and *Thelephora terrestris* ectomycorrhizae. [In process.]

This paper describes a procedure for using dry basidiospores of *Pisolithus tinctorius* for inoculating container-grown pine seedlings and the results of an initial inoculation test.

MATERIALS AND METHODS

Spencer-Lemaire Rootrainers® (Hillson model, 150 cc/cavity)[4] were filled with a mixture of vermiculite and milled pine bark (1:9 v/v) previously fumigated with methyl bromide (MC-2 at 1 kg/6 m³). Stratified loblolly pine seeds were germinated in a tray containing a mixture of sand and peat moss (1:1 v/v). Approximately 5 days after seed germination, seedlings were transplanted singly to cavities in each of 25 container units; each unit had 32 seedlings.

A specially designed chamber with two compartments was constructed of medium-density overlay plywood to accommodate a single container unit during inoculation (fig. 1). One compartment was a column measuring $64 \times 38 \times 122$ cm tall with a door in the bottom to permit the insertion of a container unit and a hole in the top for the introduction of dry spores. A 2.5-cm circular spoon (with a 20-cm wire handle) containing 200 mg of spores was lowered 10 cm into the top hole. A short blast of compressed air directed through a hose onto the spores in the spoon created a uniform cloud of basidiospores. Spores settling to the bottom of the compartment were
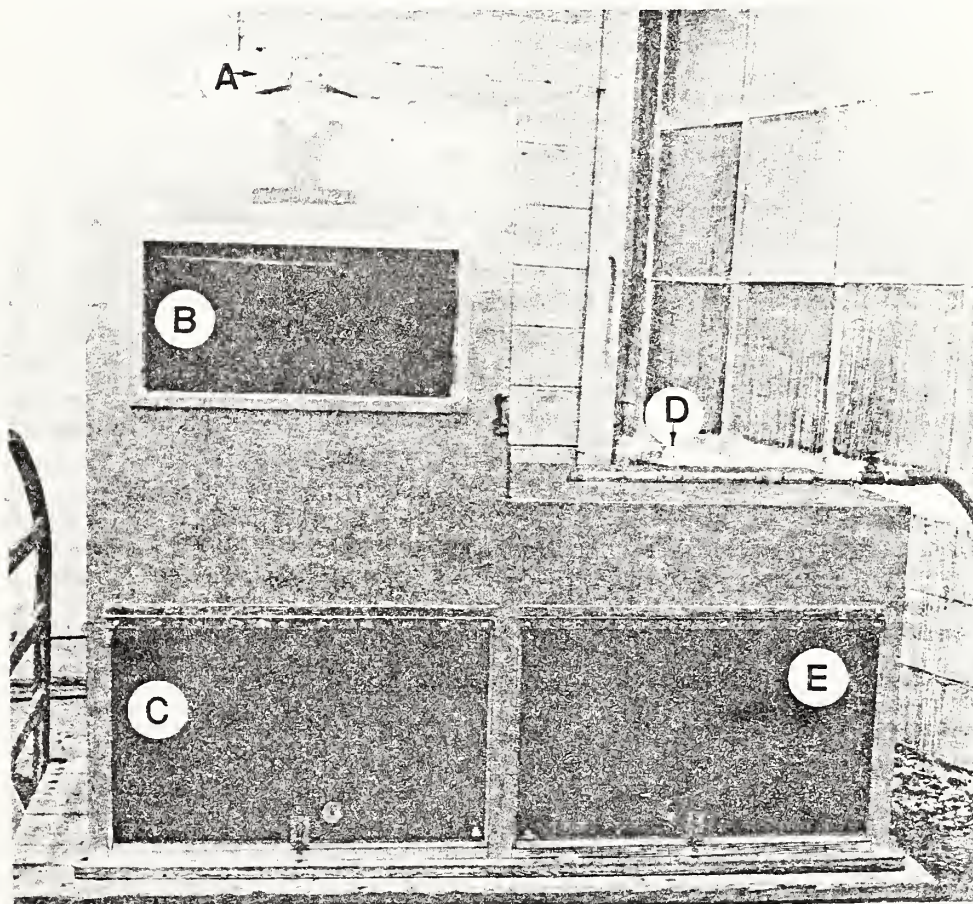
Figure 1.—Chamber used for inoculating containerized pine seedlings with spores. Tall compartment on left for inoculating with dry spores; compartment on right fitted with mist nozzles for rinsing spores into growing medium. *A*, top opening for inserting spoon containing spores; *B*, viewing window for observing release of spores; *C*, door for inserting unit in inoculation compartment; *D*, water supply to mist nozzles; *E*, door to mist compartment.

evenly deposited at a rate of approximately 75,000 spores/cm^2. Since the top opening of each container cavity was 12.3 cm^2, approximately 920,000 spores were deposited on the surface of the growing medium supporting each seedling. (Preliminary trials with smaller quantities of spores [<200 mg] resulted in an uneven deposition of spores at the bottom of the chamber.) After spore deposition, each container unit was moved to an adjoining compartment (64 × 38 × 60 cm tall) and sprayed with a water mist for 2 minutes to wash spores into the growing medium. After inoculation, container units were placed on a greenhouse bench and fertilized with a commercially available water-soluble NPK fertilizer (23:19:17) at 2,500 p/m. Approximately 30 ml of fertilizer were added to each cavity at planting and every 3 weeks thereafter. One application of a 4-percent solution of Sequestrene® 330-Fe (chelated iron 10 percent) was applied to all containers to saturation 6 weeks after transplanting to correct an FE deficiency. All seedlings were watered during the test period as needed.

Treatments consisted of inoculating seedlings 0, 2, 4, 6, or 8 weeks after transplanting. Each treatment was replicated five times; each replicate consisted of one unit of 32 seedlings. Noninoculated controls were not used in this test because naturally occurring *Pisolithus* ectomycorrhizae have never been observed in previous greenhouse tests.

Eighteen weeks after the first units were inoculated, the study was terminated. Ten randomly selected seedlings were removed from each container unit and washed free of growing medium. Seedling height and fresh weight of tops and roots were recorded. Ectomycorrhizae were visually estimated according to the procedure described by Marx and Bryan (1975).

RESULTS AND CONCLUSIONS

Height and fresh weight of tops and roots of seedlings after 18 weeks were not affected by treatments. The overall means were: Height = 17.0 cm (±0.8); top weight = 2.5 g (±0.3); root weight = 1.8 g (±0.2).

Seedlings inoculated at 0 or 2 weeks had significantly more *Pisolithus* ectomycorrhizae than those inoculated later (fig. 2). However, the highest percentage of total ectomycorrhizal development was found on seedlings inoculated at 2, 4, or 6 weeks. Naturally occurring *Thelephora*
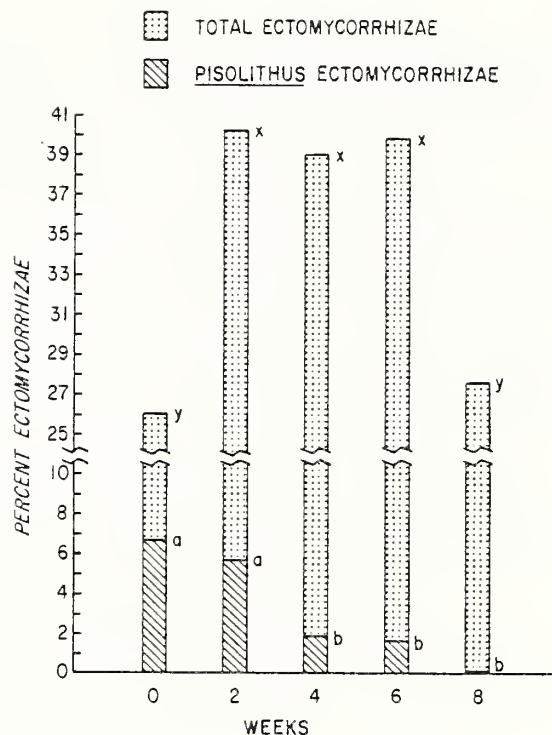
Figure 2.—Total percent of feeder roots with ectomycorrhizae formed by *Pisolithus* and *Thelephora* and percent of *Pisolithus* ectomycorrhizae formed on containerized loblolly pine seedlings inoculated with basidiospores at different time intervals. Columns with a common letter are not significantly different (P=0.05).

terrestris was the only other fungal symbiont observed on the roots. Early colonization by *Thelephora* may have accounted for the lower percentages of *Pisolithus* colonization on seedlings inoculated after 2 weeks.

Frequency of seedlings with *Pisolithus* ectomycorrhizae averaged 98 percent at 0 time, 76 percent at 2 weeks, 38 percent at 4 weeks, and 2 percent at 8 weeks. To produce ectomycorrhizae on the most seedlings, inoculation with spores should be done at the time of transplanting. If containers are direct-seeded instead of transplanted, inoculation approximately 1 week after seedling emergence would be equivalent to the 0 time of transplanting.

The average ectomycorrhizal development of seedlings by *Pisolithus* obtained in this study was too low (10 percent) to improve growth of outplanted seedlings. Outplanting trials[5] with

[5]Unpublished data, Institute for Mycorrhizal Research and Development, Athens, Georgia.

other containerized pine species indicate that 50 to 60 percent of the roots should be colonized by *Pisolithus* in order to obtain improved survival and rapid seedling growth. Additional research with spore inoculation is needed to increase the percentage of *Pisolithus* colonization of pine roots.

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Growth of Containerized Loblolly Pine with Specific Ectomycorrhizae after 2 Years on an Amended Borrow Pit

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A borrow pit with exposed subsoil in South Carolina was graded level and deep subsoiled. Plots were amended with processed sewage sludge or commercial fertilizer followed by a seeding with fescue. Container-grown loblolly pines colonized with *Pisolithus tinctorius*, *Thelephora terrestris*, or no ectomycorrhizae were planted by hand on the plots one year after site preparation.

Two years after planting on sludge-amended plots, seedlings initially colonized with *Pisolithus* had greater height, root-collar diameter, and seedling volume (D^2H) than *Thelephora* or control seedlings. The means for these three growth parameters on seedlings planted on fertilized plots were no different between *Pisolithus* and *Thelephora* seedlings, but *Pisolithus* seedlings were greater than controls. There was no difference in survival among mycorrhizal treatments on the sludge plots. Survival and seedling volume were integrated into plot volume indices (PVI). Seedlings on sludge plots had greater PVI than seedlings on fertilized plots. *Pisolithus* seedlings on sludge plots had 265 and 528% greater PVI after two years than *Thelephora* or control seedlings.

Containerized loblolly seedlings tailored with *Pisolithus* can be successfully established and rapid growth obtained on a subsoiled borrow pit amended with sewage sludge. This procedure may be applicable to thousands of acres of similar borrow pits left by highway and construction work.

INTRODUCTION

ECTOMYCORRHIZAL fungi are ubiquitous in disturbed soils of natural forests. Trees in these forests are usually colonized by symbionts ecologically adapted to the existing physical, chemical, and biological soil conditions (Mikola, 1973). Consequently, pine seedlings in recently harvested forests rarely suffer from ectomycorrhizal deficiencies. The afforestation of grasslands or adverse sites with pines, however, is usually met with failure unless the seedlings are adequately colonized by ectomycorrhizal fungi at planting time. Even nursery seedlings well colonized with symbionts often fail to survive and grow on severely disturbed sites if the fungal symbionts on the seedlings roots are not adapted

to the soil conditions on the site (Marx, 1977).

The cultural procedures used to produce pines in nurseries favor symbiotic fungi adapted to high soil moisture and fertility. *Thelephora terrestris* (Tt), one of the most common symbionts in southern nurseries, is an example. Unfortunately, the inability of this symbiont to function on harsh sites results in poor initial host survival and growth. *Pisolithus tinctorius* (Pt) is ecologically adapted to strip-mined and other disturbed sites (Schramm, 1966), and by tailoring pine seedlings in the nursery with this symbiont, tree survival and growth is improved on disturbed lands (Marx and Artman, 1979).

Borrow pits are surface-mined sites used to supply fill soil for building construction, dams, and highways. These pits, created by removing several meters of upper soil layers, often become

severely eroded and leave a gullied landscape without vegetative cover, along highways and around cities. The exposed and eroded subsoil in these pits is usually compacted, droughty, stony, and inherently low in fertility. From personal observations of several early reforestation attempts, poor survival and growth were noted where standard planting techniques were used to establish native pines on borrow pits in the piedmont region of the southern United States. New techniques of site amelioration followed by planting pines with specific ectomycorrhizae tolerant to adverse soil conditions might be combined in attempts to stabilize these sites. Deep subsoiling to fracture the compacted soil may be a useful technique (Berry, 1977). Natural forests accumulate, store, and recycle nutrients from organic matter deposited on the soil by the existing plant cover. On surface-mined areas, where these organic horizons have been removed, sewage sludge has been successfully added to initiate nutrient cycling (Smith and Evans, 1977). Pine trees with ectomycorrhizae ecologically adapted to adverse soil conditions can also be useful (Marx, 1977). These cultural practices used together in the proper sequence might succeed in correcting problems accounting for past afforestation failures on borrow pits. The use of container-grown pine seedlings instead of nursery produced bare-root seedlings may also have merit for establishing pines on borrow pits (Ruehle and Marx, 1977). The root plug developed in containers can be transplanted without any loss of ectomycorrhizae, a problem often encountered during the lifting of bare-root nursery seedlings. Containerized seedling production can also be scheduled to provide plantable size seedlings for the season with optimum rainfall and temperature.

The aim of this study was to use a combination of soil amelioration techniques and ectomycorrhizae to determine their effects on the establishment of loblolly pine (*Pinus taeda* L.) on a typical borrow pit in the piedmont of South Carolina.

METHODS AND MATERIALS

A borrow pit site was selected on the Savannah River Plant located near Aiken, South Carolina. This site was originally sandhill soil of the Eustis series with surface layers of gray sand and subsoils of very fine, compact, yellowish-red sandy clay. This pit was created during 1950–1952 by the removal of 2 to 6 meters of upper soil layers. The exposed stratum was a heavy, impervious clay characterized by extremes in particle-size distribution and low nutrient content. Loblolly pine seedlings were planted on this pit in 1953 following standard procedures. After 22 years' growth, the trees were only 5 to 15 cm in root-collar diameter and 2.5 to 5 m tall.

In June 1975, all trees were removed and the site was leveled by grading. Two months later the site was subsoiled to a depth of 1 m on 1.2 m centers in both north-south and east-west directions. The dry conditions at this time resulted in excellent fracturing of the compacted soil. The site was then double disked to break clods and smooth ridges created by the subsoiler.

In September, 30 plots each 7.3 × 7.3 m were arranged in two rows across the site with a 6-m buffer zone separating all plots. Processed sewage sludge from Athens, Georgia, was broadcast over the soil in 15 plots at the rate of 0.9 m³/plot (approximately 1.3 cm deep). The remaining 15 plots, referred to hereafter as fertilizer plots, received broadcast applications of 560 kg/ha of commercial 10-10-10 fertilizer and 2,240 kg/ha of dolomitic limestone. All plots were double disked 10 to 15 cm deep to incorporate the amendments. The perimeter of each plot was disked to form a ridge to prevent loss of sludge and fertilizer. Three weeks later the entire study area was sown with fescue [*Festuca arundinacea* Schreb. (Ky 31)] at a rate of 30 kg/ha to control erosion and retard nutrient loss until the pine seedlings were planted the following year.

Containerized loblolly pine seedlings were produced in a greenhouse in Athens, Georgia. A growing medium composed of a peatmoss-vermiculite mixture (1:1 v/v) was mixed with pulverized 10-10-10 commercial fertilizer (9 g/100 l of growing medium). Inocula of Pt (isolate 138) and Tt (isolate 201) were produced in peatmoss-vermiculite-nutrient medium and processed as reported earlier (Marx and Bryan, 1975). Inoculum was mixed in a 1:6 v/v ratio with the growing medium. Autoclaved inoculum of Pt was mixed with growing medium at the same rate for the control seedlings. The two groups of Styroblock 8® containers* filled with medium and viable inoculum were placed in the greenhouse and the control group of containers with medium and sterile inoculum were placed in a filtered-air growth room (Marx and Bryan, 1969) to maintain seedlings in a nonmycorrhizal condition. In July 1976, all units were seeded with several seeds per cavity and, after seed germination, seedlings

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*The use of trade names in this publication is for the information and convenience of the reader, and does not constitute an official endorsement by the U.S. Department of Agriculture or the Forest Service.

Table 1. Survival and Growth of Containerized Loblolly Pine Seedlings after Two Years on a Borrow Pit in South Carolina*

Amendment	Mycorrhizal condition	Survival %	Height cm	Root-collar diameter cm	Seedling volume† cm³
Sludge	*Pisolithus* (Pt)	91.2 a‡	107.2 a	3.0 a	1215.4 a
	Thelephora (Tt)	73.6 a	76.0 b	1.9 b	390.6 b
	Control	68.0 a	70.7 b	1.6 b	236.5 b
	X̄	77.6	81.1	2.2	614.2
Fertilizer	*Pisolithus* (Pt)	96.0 a	34.5 a	0.9 a	38.0 a
	Thelephora (Tt)	88.0 b	31.4 ab	0.9 ab	35.0 ab
	Control	89.6 b	26.3 b	0.7 b	16.0 b
	X̄	91.2	30.7**	0.8**	29.7**

*Means of survivors from 25 test seedlings initially planted in each of five plots. Each number followed by a common letter within groups of parameters is not significantly different at the $p=0.05$ confidence level.

†Seedling volume (cm³) = (root-collar diameter)² × height.

**Denotes significant differences ($p=0.01$) between groups according to Student's t-test.

were thinned to one per cavity. All seedlings were watered as needed with tap water. No additional fertilizer was added. Additional seedlings were grown in the greenhouse in styroblocks containing only growing medium to provide seedlings for the border rows.

In November 1977, ten randomly selected seedlings per treatment replicate were measured and their roots were visually examined for ectomycorrhizae. Inoculated seedlings, both Pt and Tt, averaged 14.7 cm in height and 2.1 mm in root-collar diameter. Control seedlings averaged 13.1 cm in height and 1.9 mm in root-collar diameter. Seedlings were hand planted in each plot in five rows of five seedlings each on 1.2 m centers. A border row was planted around each plot. The six treatments, replicated five times, were assigned to plots at random.

Biomass samples were collected from the ground cover on each plot at planting time and after each of the next two growing seasons. Each plot of 25 test seedlings created a grid containing 16 squares measuring 1.2 m². Three squares were selected at random at each sampling period and a 0.3-m² frame was placed in the center of each. All grass within each frame was clipped by hand to within 1 cm of the ground. All clippings from each plot were combined in a tared paper bag, ovendried at 90°C for 72 h, and weighed.

In November 1978, trees in all plots were measured for root-collar diameter and height. Five randomly selected trees per replicate were dug from plots within each treatment to visually assess ectomycorrhizal development. Soil samples were removed to 10 cm depth from each plot, air dried at room temperature for 10 days, and processed for nutrients by the 0.05N HCl +

0.02N H_2SO_4 extraction and soil organic matter was determined by the Walkely-Black wet oxidation method (Wells et al., 1973). Foliar samples of current-year needle bundles were removed from five randomly selected trees per plot, combined into one sample, and dried at 90°C for 72 h prior to tissue analysis for total N by Kjeldahl and for the other elements by dry ash methods (Wells et al., 1973). Concentrations of phosphorus were determined colorimetrically and all other ions were assayed by atomic absorption. Analyses of foliar and soil samples were performed by Carol G. Wells, U.S. Department of Agriculture, Forest Service, Forestry Services Laboratory, Research Triangle Park, North Carolina.

Analyses of variance were made on all data following procedures for a completely random design, and treatment differences were evaluated with Duncan's Multiple Range Test (P = 0.05) and combined group means were tested with Student's t-test (P = 0.01).

RESULTS

Seedlings with Pt ectomycorrhizae at planting had significantly better survival at the end of the study than seedlings colonized with Tt or non-mycorrhizal seedlings in the fertilized plots (Table 1). Survival of seedlings on sludge-amended plots was not significantly affected by ectomycorrhizal treatment. Dense grass and deer bedding caused considerable mortality of pine seedlings in sludge plots, with damage by deer causing greater within-treatment variation in seedling survival than in the fertilizer plots.
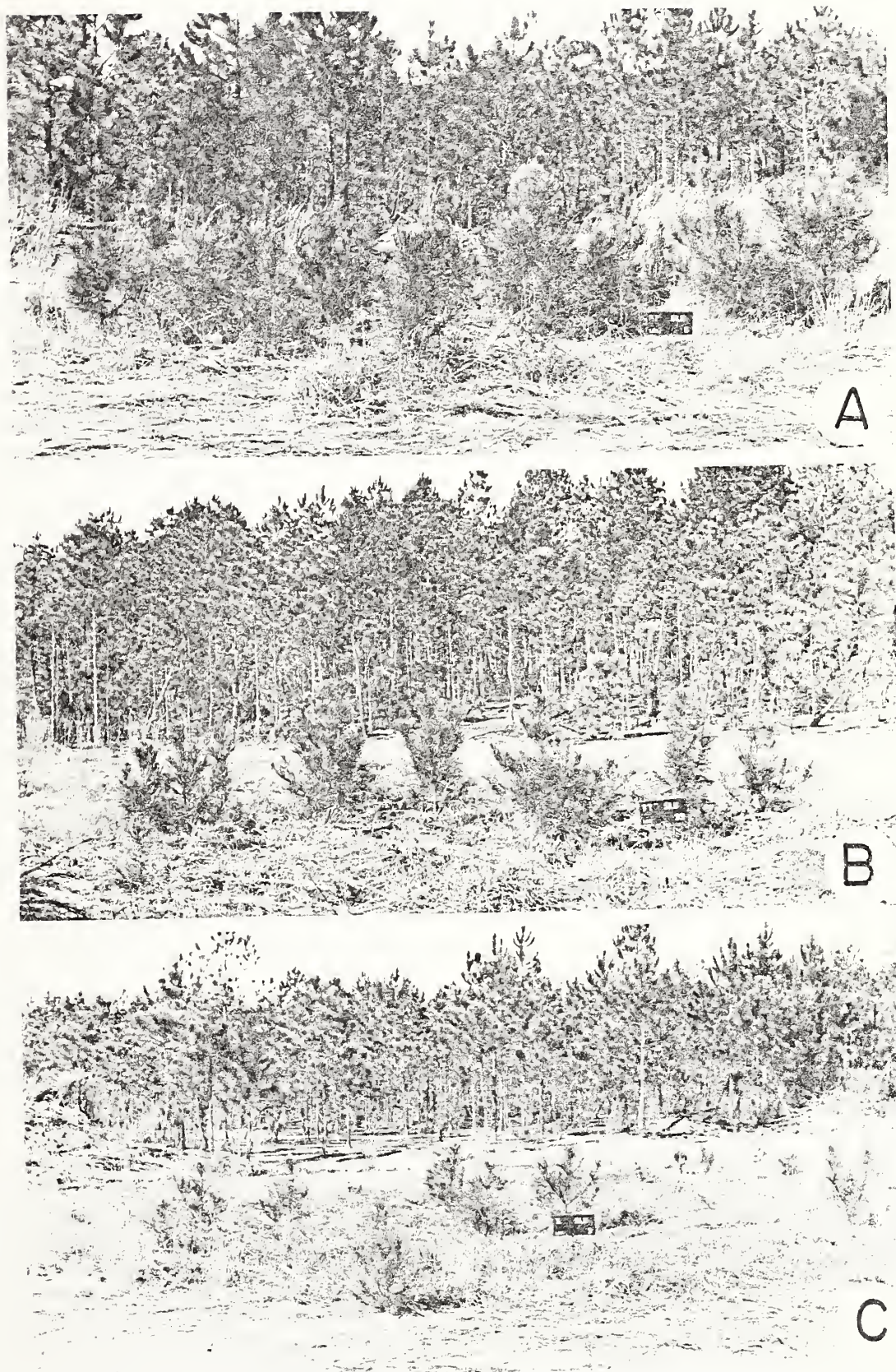
In sludge plots, seedlings with Pt ectomycor-

Figure 1. Two-year growth of containerized loblolly pine seedlings colonized with specific ectomycorrhizal fungi and planted in borrow pit plots amended with sewage sludge. A—Pisolithus tinctorius, B—Thelephora terrestris. C—control (nonmycorrhizal).

rhizae at planting had significantly greater height, root-collar diameter, and seedling volume than seedlings with Tt ectomycorrhizae or with none (Table 1, Fig. 1). The average volume of seedlings with Pt ectomycorrhizae was over 200% greater than seedlings with Tt and over 400% greater than seedlings initially without ectomycorrhizae. In the fertilizer plots, seedlings with Pt ectomycorrhizae were not significantly larger than seedlings with Tt ectomycorrhizae, but they were significantly greater in height, root-collar diameter, and seedling volume than seedlings lacking ectomycorrhizae at planting.

Survival and growth data were integrated into plot volume indices (PVI) (Marx et al., 1977) and the same growth differences due to treatments were noted (Fig. 2). In the sludge plots, seedlings with ectomycorrhizae at planting had PVI that were 265 and 528% greater than seedlings with Tt ectomycorrhizae or no mycorrhizae. In the fertilizer plots, PVI of seedlings with Pt were no different from those with Tt, but they were significantly greater by 158% than those of nonmycorrhizal seedlings. As a group, seedlings on sludge plots had 900% greater PVI than those on fertilizer plots.

Chemical analyses of soil showed that major elements and organic matter were low in the fertilizer plots by the end of the study (Table 2). There was significantly more organic matter, N, and P, and less Mg in sludge-amended plots than in fertilizer plots. Sludge plots average 1 pH unit lower than fertilized and limed plots. Seedlings with different ectomycorrhizal treatments did not affect the chemical status of the soil. Needles from seedlings in sludge plots had more N, Mn, Na, and Zn than those from seedlings in fertilizer plots (Table 3). However, needles from seedlings in fertilizer plots had more Al, Ca, Fe, and Mg than needles from seedlings in sludge plots. Although there were differences in metal ion concentrations in the foliage of seedlings from sludge amended plots versus the fertilized plots, there were no symptoms of metal toxicity. Needle concentrations of P, K, and Cu were not affected by soil amendments. In the sludge plots, seedlings with Pt ectomycorrhizae at planting had less foliar N at the end of the study than those with Tt ectomycorrhizae or with none. Ectomycorrhizal condition of seedlings failed to affect the foliar concentrations of other elements assayed.

Root evaluations of ectomycorrhizae on seedlings after two years revealed that Pt was dominant on seedlings in all plots planted with Pt seedlings, and also accounted for 20% of all the ectomycorrhizae on seedlings initially with Tt ectomycorrhizae and about 10% of the ectomycor-
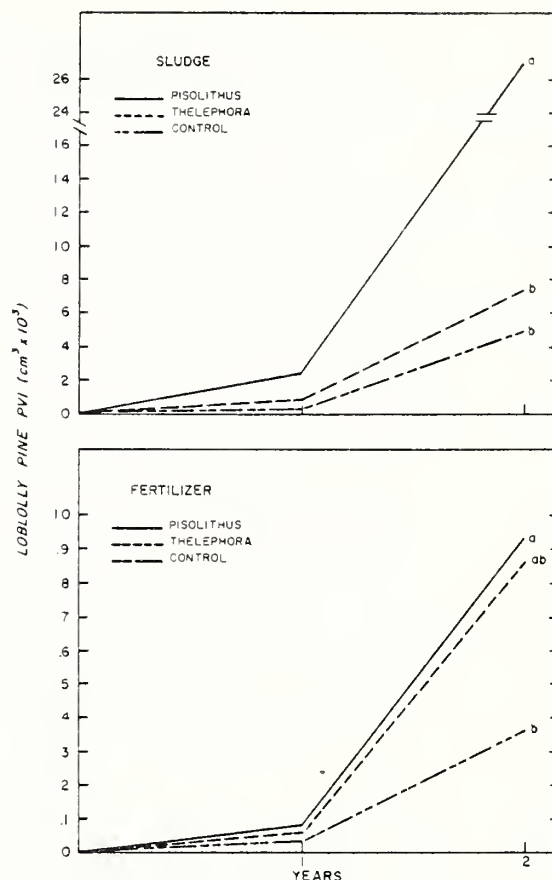

Figure 2. Plot volume indices (PVI) of loblolly pine seedlings with Pisolithus, Thelephora, *or no ectomycorrhizae at planting after two years in amended plots on a borrow pit in South Carolina. PVI = (root-collar diameter)^2 × height × number of surviving seedlings per plot. Points within each fertility treatment at the two-year line followed by the same letter are not significantly different (P = 0.05). Note the difference in the scale between sludge and fertilizer treatments.*

rhizae on seedlings initially without ectomycorrhizae. Tt constituted about 70% of all the ectomycorrhizae on seedlings with Tt at planting and nearly 50% of the ectomycorrhizae on those without ectomycorrhizae at planting. An unidentified white symbiont accounted for nearly 25% of the ectomycorrhizae on seedlings in all plots.

Ground cover biomass on sludge plots averaged 468 and 510 g/m^2, respectively, for 1977 and 1978. Biomass on fertilized plots was markedly less than that on sludge plots averaging 69 and 33 g/m^2 for these two years.

DISCUSSION

Containerized loblolly seedlings can be successfully used to establish trees on a borrow pit. Overall survival on both sludge and fertilized

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*Table 2. Chemical Soil Properties on Plots in the Borrow Pit in South Carolina Three Years after Addition of Amendments**

| Amendments | Mycorrhizal condition | ppm | | | | | Organic matter % | pH |
		N	P	K	Ca	Mg		
Sludge	*Pisolithus*	523 b†	59 b	7 a	17 a	11 a	1.68 b	4.0 a
	Thelephora	583 b	72 b	9 a	17 a	10 a	1.60 b	3.8 a
	Control	581 b	59 b	10 a	19 a	13 a	1.83 b	3.8 a
	X̄	562.5	63.3	8.8	17.7	11.2	1.71	3.84
Fertilizer	*Pisolithus*	109 a	4 a	8 a	13 a	58 b	0.49 a	4.8 b
	Thelephora	128 a	6 a	7 a	13 a	60 b	0.48 a	4.9 b
	Control	103 a	10 a	8 a	16 a	72 b	0.46 a	4.9 b
	X̄	127.6‡	6.6‡	7.8‡	14.1‡	63.4‡	0.48‡	4.85‡

*Values are means of five samples, and for the elements, represent extractable fractions.
†Means within columns not followed by the same letter are significantly different $(p = 0.05)$.
‡Denotes significant differences between group means $(p = 0.01)$ according to Student's t-test.

*Table 3. Foliar Analysis of Containerized Loblolly Pine Seedlings after Two Years on a Borrow Pit in South Carolina**

| Amendment | Mycorrhizal condition | % | | | | | ppm | | | | | |
		N	P	K	Ca	Mg	Mn	Fe	Na	Zn	Cu	Al
Sludge	*Pisolithus*	1.65 b†	0.15 a	0.34 a	0.22 a	0.07 a	256 bcd	40 a	123 a	150 b	5 a	651 a
	Thelephora	1.95 c	0.15 a	0.47 b	0.29 ab	0.10 a	372 cd	49 a	103 a	150 b	4 a	689 a
	Control	1.89 c	0.18 a	0.49 b	0.28 ab	0.10 a	313 d	51 a	103 a	146 b	4 a	802 ab
	X̄	1.832	0.162	0.435	0.261	0.091	313.9	46.7	109.8	148.5	3.2	713.8
Fertilizer	*Pisolithus*	1.51 ab	0.13 a	0.42 ab	0.34 b	0 21 b	104 a	106 b	54 a	46 a	3 a	1061 c
	Telephora	1.52 ab	0.14 a	0.34 a	0.32 b	0.19 b	121 ab	146 b	84 a	60 a	4 a	1079 c
	Control	1.49 a	0.14 a	0.43 ab	0.34 b	0.18 b	163 abc	122 b	84 a	61 a	3 a	991 bc
	X̄	1.523‡	0.137	0.395	0.334‡	0.193‡	129.4‡	124.9‡	74.1‡	55.8‡	3.2	1043.6‡

*Values are means of five samples.
†Means within columns not followed by the same letter are significantly different $(p = 0.05)$.
‡Denotes significant differences between group means $(p = 0.01)$ according to Student's t-test.

plots was as good or better than comparable bare-root seedlings in an adjoining study on this borrow pit (Berry and Marx, 1980). The size of container seedlings was slightly less than comparable bare-root seedlings after two years, i.e., seedling volume on sludge/Pt plots averaged 1,215 cm³ for container seedlings and 1,885 for bare-root seedlings. The smaller seedling size for container seedlings at planting probably accounted for this smaller volume.

Although the Pt treatment accounted for a marked increase in survival and tree growth on fertilizer plots over the control, even trees with this symbiont were considerably smaller after two years than trees initially without ectomycorrhizae on sludge plots. Although nutrient levels in fertilizer plots were considerably lower at the end of the study than those in sludge plots, they were still not below levels normally encountered in many southern forest soils (Pritchett and

Smith, 1970). At favorable temperature and with adequate soil nutrients, root growth of loblolly pine is controlled primarily by soil moisture and aeration (Bilan, 1968). Sludge treatments in this study maintained a moister and better aerated soil than fertilizer treatments. Soil in sludge plots compared to fertilized plots had more cellulose-degrading fungi, slime molds, and larger numbers of yeasts and bacteria.* These observations suggest that processes of mineral recycling are more advanced after three years in borrow pit soil amended with sludge than soil amended with fertilizer. The well-developed grass biomass on sludge plots also shaded the soil to maintain lower surface temperatures and probably allowed better pine root development in the

*Wojcik, V. H. and D. H. Marx. Unpublished data. Southeast. For. Exp. Stn., Forestry Sciences Laboratory, Athens, Georgia.

upper layers during the summer months. Foliar analysis indicated that levels of N, P, and K were satisfactory in the needles of seedlings in both sludge and fertilizer plots (Wells, 1970; Wells et al., 1973). In sludge plots, the lower concentrations of foliar N in *Pisolithus* seedlings was probably a reflection of dilution effect caused by greater top growth compared to *Thelephora* and nonmycorrhizal seedlings. This concentration was still well above amounts found in foliage of loblolly on fertilized forest soils. Therefore, poor physical properties of soil in fertilizer plots probably accounted for poorer root development and less overall growth of seedlings in these plots. The excellent two-year growth of loblolly pine in the sludge plots reflect the improvements to soil fertility and soil moisture provided by sludge amendments.

Grass cover that developed well in sludge plots provided other benefits to borrow pit soil. A portion of the nutrients released from the sludge were taken up by the grass and provided a nutrient reserve in the system. This cover provided adequate soil stabilization for erosion control. Although this borrow pit was graded level and erosion was not a serious problem, if there had been a slope on this site the fertilized plots with little grass cover would have undergone serious erosion.

The poorer growth of seedlings with no mycorrhizae at planting indicates that seedlings with any ectomycorrhizae at planting are better than those with no ectomycorrhizae. Containerization programs should adjust cultural procedures to ensure ectomycorrhizal development on pine seedlings. The improved growth of seedlings with Pt ectomycorrhizae demonstrates the point made by Marx (1977) that certain species of ectomycorrhizal fungi are more beneficial to pines than others, particularly on stressed sites.

Amelioration of any borrow pit physically and chemically unsuited for supporting vegetation probably will require the integration of both cultural and vegetative methods: (1) subsoiling to fracture the indurate soil surface layers, (2) addition of appropriate organic matter to restore necessary physical and biological factors, and (3) a combination of grass cover and forest tree seedlings colonized with beneficial mycorrhizal symbionts ecologically adapted to adverse sites. Such a unified program for stabilizing borrow pits is the key to rapid establishment of a self-perpetuating vegetative cover which can return these nonproductive biological deserts into productive land for trees, wildlife, and water management schemes.

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Survival and Growth of Container-Grown and Bare-Root Shortleaf Pine Seedlings With *Pisolithus* and *Thelephora* Ectomycorrhizae

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ABSTRACT. Bare-root and container-grown shortleaf pine (Pinus echinata Mill.) seedlings with Pisolithus tinctorius and Thelephora terrestris ectomycorrhizae were outplanted on two reforestation sites on the Ouachita National Forest. On the better site, survival and growth of containerized seedlings were better than that of bare-root seedlings. On the poorer site, the reverse was true. Mycorrhizal treatment provided no consistent advantage for survival and growth for containerized seedlings. However, bare-root seedlings with half or more of their ectomycorrhizae formed by P. tinctorius before planting had greater survival and growth on both sites than seedlings with lesser amounts of Pisolithus ectomycorrhizae. The difference in mycorrhizal treatments among containerized and bare-root seedlings are discussed.

Shortleaf pine is well adapted to the dry ridges of the Ouachita and Kiamichi Mountains of western Arkansas and eastern Oklahoma (Wakeley 1954). The Ouachita National Forest encompasses many acres in these mountains, and after compartment clearcuts, artificial regeneration is usually preferred over natural regeneration. Recent planting failures with nursery-run shortleaf pine seedlings have stimulated local forest managers to look for innovative means to improve artificial regeneration. Investigations are needed for ectomycorrhizal inoculation and containerization as two possible alternatives to traditional regeneration procedures.

Containerization often has advantages over bare-root production by improving survival and growth of certain species, making more efficient use of quality seed, and gaining an extended planting season which allows rapid adjustment of seedling production to meet fluctuating demands (Balmer 1974). Recent research has shown that container-grown pines can be inoculated with specific ectomycorrhizal fungi (Marx and Barnett 1974, Ruehle and Marx 1977). Inoculating nursery beds and

containerized shortleaf pine seedlings with selected mycorrhizal symbionts ecologically adapted to the planting site may offer an attractive possibility for improving survival of short-leaf pine seedlings. Different amounts of ectomycorrhizae formed by various fungi have had significant effects on survival and growth of various pine species planted on reforestation (Marx et al. 1977) and adverse sites (Marx 1977, Marx and Artman 1979) in the South.

The objective of this experiment installed on two reforestation sites on the Ouachita National Forest was twofold: (1) to compare the field performance of bare-root shortleaf pine seedlings having different amounts of *Pisolithus tinctorius* and *Thelephora terrestris* ectomycorrhizae and container-grown shortleaf seedlings having the same amounts of either *Pisolithus* or *Thelephora* ectomycorrhizae, and (2) to determine the field performance of bare-root seedlings compared with container-grown seedlings having comparable ectomycorrhizal treatments.

MATERIALS AND METHODS

Inoculum Production

Mycelial inocula of *Pisolithus tinctorius* (Pt) [Isolate 138] and *Thelephora terrestris* (Tt) [Isolate 201] were produced and leached as previously reported (Marx and Bryan 1975). The inocula were stored in plastic bags at 38°F for four days before use.

Production of Bare-root Seedlings

In the spring of 1976, inoculum of *P. tinctorius* was broadcast on methyl bromide-fumigated (MC-2) soil at the W. W. Ashe Nursery, USDA Forest

Service, near Brooklyn, Mississippi, and mechanically incorporated into the upper 4 inches of soil (Marx et al. 1976). Stratified and Arasan®-treated seed of shortleaf pine from a mixed lot collection made in one location on the Ouachita National Forest, Arkansas, were sown. *Thelephora terrestris* was not artificially introduced into soil at this nursery because it occurs naturally in great abundance.[1]

In mid-December 1976, all seedlings were undercut with a root pruning bar to a depth of 8 inches, hand lifted, and graded. Seedlings less than 5 inches tall or with root collar diameter less than one-eighth inch were discarded as culls. Seedlings of plantable size were graded visually for *Pisolithus* or *Thelephora* ectomycorrhizae according to groups: High Pt: Low Tt = 65 percent Pt and 10 percent Tt; Medium Pt and Tt = 35 percent Pt and 40 percent Tt; Low Pt and High Tt = 10 percent Pt and 65 percent Tt; and control (all Tt) = 75 percent Tt. Seedling height and root collar diameter averaged 9.2 ($\pm$0.7) and 0.20 ($\pm$0.05) inches, regardless of ectomycorrhizal group. Roots were sprayed with kaolin slurry, and seedlings were packaged in polyethylene-lined kraft paper bags, and stored at 38°F until planted.

Production of Containerized Seedlings

In May 1976, inoculum of Pt or Tt was mixed with milled pine bark (fine grade) at a rate of 1:8 v/v. Sterilized inoculum of *P. tinctorius* was mixed at the same rate for the two sets of control seedlings. Styroblock-4® container units were filled, watered with a diluted wetting agent (Aqua-gro®), and seeded with Arasan®-coated shortleaf pine seed. The seed used for container production were from the same lot used for bare-root production. The container units were placed in a greenhouse located at the Alexandria Forestry Center, USDA Forest Service, Pineville, Louisiana. Two weeks after germination, seedlings in treatments 1 (Pt), 2 (Tt), and 3 (control) were fertilized every three weeks with a water-soluble NPK fertilizer (20:19:18) diluted to 2500 ppm and applied to saturation. Seedlings in treatment 4 (high-fertility control) were fertilized every week. In September, fertilization was terminated and seedlings were moved to a shadehouse to harden off. Several randomly selected seedlings per treatment were measured and assessed for ectomycorrhizal development in mid-October. Heights of seedlings in treatments 1, 2, and 3 averaged 4.1 $\pm$ 0.3 inches and those in treatment 4 averaged 7.0 $\pm$ 0.2 inches. Root collar diameter in the first three treatments aver-

aged 0.07 $\pm$ 0.004 inches and those in treatment 4 averaged 0.10 $\pm$ 0.003 inches. Ectomycorrhizae averaged 61 percent Pt and 21 percent naturally occurring Tt in treatment 1; 56 percent Tt in treatment 2; 26 percent naturally occurring Tt in treatment 3; and 16 percent naturally occurring Tt in treatment 4.

Plot Installation

Two sites were selected on the Ouachita National Forest. Site 1, in the Mena District just north of Mena, Arkansas, was a mixed pine-oak stand recently clearcut and the slash burned in place. The soil was a loam overlying a silty clay subsoil in the Carnasaw series and had good characteristics for shortleaf pine growth. Site 2, in the Choctaw District near Page, Oklahoma, was a ridgetop site that also had been recently clearcut of mixed pine-oak and the slash burned. The soil was also a Carnasaw loam but had a drier moisture regime.

Twenty plots, each measuring 32 × 64 feet, were arranged in five blocks in a 2.5-acre area on each site for the bare-root seedlings. Twenty additional plots were arranged the same way in an adjacent 2.5-acre area on each site for container seedlings. Each plot had seven rows spaced 8 feet apart with 7 seedlings spaced 4 feet apart in the row. A border row of nursery-run shortleaf pine seedlings was planted around each plot. All plots were separated by 10-foot nonplanted strips. Treatments were randomly assigned to plots within blocks. In late October 1976, container seedlings were hand-planted by using bullet-shaped planting tools. Bare-root seedlings were planted by hand in January 1977.

Evaluation of Seedlings

At the end of the first and second growing seasons seedlings were measured for height and root collar diameter. At the end of the second growing season five randomly selected seedlings per plot were excavated and roots assessed visually on site for ectomycorrhizal development. All data were subjected to analyses of variance. Significant means were separated using Duncan's Multiple Range Test.

RESULTS

Containerized seedlings had better survival and growth than bare-root seedlings on site 1 (Table 1); the reverse was true on site 2 (Table 2). Overall survival of containerized seedlings was 59 percent better on site 1 than on site 2. Survival of bare-root seedlings was also better on site 1. On site 2, grass, oak sprouts, brambles, forbs and natural

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[1] Mention of commercial products in this paper does not constitute endorsement by the U.S. Department of Agriculture to the exclusion of others that might be suitable.

Table 1. Survival and growth of bare-root and containerized shortleaf seedlings with *Pisolithus* (Pt) and *Thelephora* (Tt) ectomycorrhizae after two years on site 1 near Mena, Arkansas.[1]

Ectomycorrhizal condition at planting of seedlings	Survival	Height	Root collar diameter	Seedling volume[2]	PVI[3]
			Container		
	Percent	*Feet*	*Inches*	............Cubic Inches	
Pt—low fertility	86.9a	1.7a	0.32b	2.8b	117.8b
Tt—low fertility	84.9a	1.8a	0.37ab	4.3ab	180.0ab
Natural Tt—low fertility	81.2a	1.8a	0.35b	3.3b	133.0b
Natural Tt—high fertility	87.3a	1.9a	0.43a	5.7a	245.9a
$\bar{X}$	85.1	1.79	0.37	4.03	169.18
			Bare-root		
High Pt:low Tt	75.9a	1.7a	0.33a	2.3ab	89.7a
Medium Pt and Tt	70.2a	1.7a	0.35a	2.7a	95.2a
Low Pt:high Tt	57.1b	1.5b	0.25b	1.1c	33.6b
Control (all Tt)	56.8b	1.6ab	0.29ab	1.7b	45.8b
$\bar{X}$	65.0	1.61	0.30	1.95	66.08

[1] *Means in columns within seedling type followed by the same letter do not differ (P = .05) according to Duncan's Multiple Range Test.*
[2] *Seedling volume (in³) = (root collar diameter)² × height.*
[3] *PVI [plot volume index (in³)] = mean seedling volume × number of surviving trees per plot.*

pine regeneration were dense and caused considerable overtopping and competition to the planted pines, which probably contributed to the higher seedling mortality. The overall Plot Volume Index (PVI) for container-grown seedlings was over 150 percent greater than for bare-root seedlings on site 1. However, on site 2, where there was more competition, the PVI for bare-root seedlings was 77 percent greater than the container-grown seedlings.

Among the container-grown seedlings, mycorrhizal treatment provided no advantage to survival or growth. Yet, bare-root seedlings with the greatest amounts of *Pisolithus* ectomycorrhizae at plant-

ing had greater survival and growth on both sites than seedlings with smaller amounts of *Pisolithus* ectomycorrhizae. Root assessments after two growing seasons revealed that indigenous ectomycorrhizal fungi were quite prevalent on seedlings on both sites. A white fungal symbiont (species unknown) was commonly found on the roots of seedlings on both sites. In many cases, container-produced control seedlings at high fertility having few naturally occurring Tt ectomycorrhizae at planting were found to be colonized with this white symbiont both on new roots and those roots located in the original planting plug. The unidentified white fungal symbiont was also observed on roots

Table 2. Survival and growth of bare-root and containerized shortleaf pine seedlings with *Pisolithus* (Pt) and *Thelephora* (Tt) ectomycorrhizae after two years on site 2 near Page, Oklahoma.[1]

Ectomycorrhizal condition at planting of seedlings	Survival	Height	Root collar diameter	Seedling volume[2]	PVI[3]
			Container		
	Percent	*Feet*	*Inches*	............Cubic Inches	
Pt—low fertility	55.9a	1.5a	0.24a	1.5a	42.1a
Tt—low fertility	48.6a	1.7a	0.28a	2.4a	58.6a
Natural Tt—low fertility	49.0a	1.5a	0.24a	1.4a	34.8a
Natural Tt—high fertility	46.5a	1.6a	0.28a	1.9a	45.8a
$\bar{X}$	50.0	1.58	0.26	1.80	45.33
			Bare-root		
High Pt:low Tt	64.5a	1.9a	0.37a	3.5a	110.5a
Medium Pt and Tt	65.3a	2.0a	0.37a	3.6a	115.9a
Low Pt:high Tt	50.6ab	1.7a	0.30b	1.8b	47.0b
Control (all Tt)	46.5b	1.7a	0.31a	2.0b	45.8b
$\bar{X}$	56.7	1.83	0.34	2.73	78.80

[1] *Means in columns within seedling type followed by the same letter do not differ (P = .05) according to Duncan's Multiple Range Test.*
[2] *Seedling volume (in³) = (root collar diameter)² × height.*
[3] *PVI [plot volume index (in³)] = mean seedling volume × number of surviving trees per plot.*

of oak sprouts and volunteer pine seedlings in the plots. This fungus undoubtedly spread from these roots to those of the test seedlings. Container-grown seedlings of the Pt treatment had persistent *Pisolithus* within the plug, but few feeder roots emerging from the original plug were colonized with Pt. *Pisolithus* readily spread to new roots formed after outplanting on most bare-root seedlings.

DISCUSSION AND CONCLUSIONS

Even though indigenous ectomycorrhizal fungi were prevalent on these reforestation sites, bare-root shortleaf pine seedlings survived and grew better if they had half or more of their ectomycorrhizae formed by *Pisolithus* before planting. This agrees with earlier findings for other pine species on similar reforestation sites (Marx *et al.* 1977). Because of their relatively small size, containerized shortleaf pine seedlings were more sensitive to competition than the larger bare-root seedlings. The use of container-grown seedlings of this size may require more thorough site preparation to reduce first-year competition. Also, large container-grown seedlings may be more comparable to bare-root seedlings on sites with this much competition.

Among the containerized shortleaf pine the lack of difference in survival resulting from mycorrhizal treatment in this study agrees with the findings of Marx and Barnett (1974) for containerized loblolly pine seedlings planted on sites in Louisiana. However, other studies (unpublished data) on field performance of containerized pine seedlings on stressed sites, such as coal spoils or amended borrow pits, show that seedlings with abundant *Pisolithus* ectomycorrhizae (>40 percent) before outplanting survive and grow faster than seedlings with *Thelephora* or no ectomycorrhizae.

Bare-root seedlings of southern pines have a dominant main root and lateral roots that spread horizontally and extend several times the height of the seedlings within a few months after planting. This growth pattern was not apparent with container-grown seedlings in this study. When developing roots are confined with containers and modified by air pruning, the root system is transformed into a dense mat of vertically oriented fibrous roots molded into a plug the size and shape of the container. With this root configuration, new lateral roots extend into the soil surrounding the planting hole in a more vertical orientation, at least initially, than those of bare-root seedlings.

Since we observed that new lateral roots of container-grown seedlings grew deeper in the soil on these sites than those of bare-root seedlings,

their microenvironment and soil associates might have been less favorable for the development of *Pisolithus* ectomycorrhizae. Correlated with this rooting habit is that, under reasonably good conditions for seedling growth, rapidly growing pine roots may outgrow the ectomycorrhizal fungi located on older parts of the root system (Bowen and Theodorou 1973). If lateral root growth extends faster or with a different orientation from a root plug, natural colonization of feeder roots by indigenous ectomycorrhizal fungi can develop and thus minimize the effect of prior inoculation. Additional research will provide answers to the question of root extension after planting and how this affects the spread of ectomycorrhizal fungi from root plugs to newly emerging feeder roots.

In conclusion, the results of this study suggest: (1) nursery inoculation with *Pisolithus tinctorius* benefits shortleaf pine regeneration using bare-root seedlings colonized with abundant ectomycorrhizae; (2) more research is needed on ectomycorrhizal container-grown seedlings; and (3) use of container-grown seedlings requires more intensive site preparation than bare-root seedlings.

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SUBSOILING IMPROVES GROWTH OF PINE ON A GEORGIA PIEDMONT SITE

by Charles R. Berry[1]

ABSTRACT.—Shortleaf and loblolly pine seedlings were planted on an eroded Georgia Piedmont site. Subsoiling with furrows 0.8 m apart and 0.6 m to 0.8 m deep significantly increased volume growth of both pine species after 5 years. Applying pine bark to a depth of 8 cm, sowing *Lespedeza sericea*, or interplanting with black locust did not influence growth of either pine species.

Keywords: Eroded sites, reforestation, *Pinus taeda*, *Pinus echinata*.

Deep subsoiling—ripping 2 feet or more in depth—has been recommended for preparing some sites for planting of tree seedlings. Sites needing such treatment and the specific benefits of treatment, however, are not well defined. Moehring (1970) recommended subsoiling to alleviate compaction damage and to break up shallow hardpans and impervious subsoil layers. Wilson (1969) advocated such treatment for soils in New Zealand, and Page (1977a, 1977b) compared subsoiler designs for use in New Zealand. Schroder (1975) found that although effects of subsoiling persisted for up to 2 years, effective field moisture capacity was not improved. Guild (1971) showed that subsoiling in New Zealand improved survival and, in some cases, height growth of planted *Pinus radiata* D. Don.

Most severely eroded sites, like those common in the Georgia Piedmont or sites otherwise devastated, such as borrow pits, require some mechanical cultivation and the addition of organic or inorganic amendments before they can be effectively reforested. On many such sites it is difficult to plant tree seedlings unless the soil has been loosened in some manner. The soil sometimes can be loosened to a minimum degree by machine-planting equipment. Subsoiling or ripping, however, improves water penetration and internal structure of many soils and facilitates planting, whether by machine or by hand.

The most serious disease of shortleaf pine (*Pinus echinata* Mill.), "littleleaf", also affects loblolly pine

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(*P. taeda* L.). Littleleaf is found almost exclusively on eroded or otherwise disturbed sites and is severe on at least 2 million hectares in the Piedmont regions of Alabama, Georgia, and South Carolina (Zak 1961). Although a fungus root pathogen, *Phytophthora cinnamomi* Rands, was indicted as a causal agent (Campbell and Copeland 1954), typical littleleaf sites are generally unfavorable for good growth of trees even when the pathogen cannot be detected (Zak 1961). Most littleleaf sites have a thin or absent topsoil and a poorly drained subsoil. Although littleleaf trees do not generally exhibit symptoms until about 15 to 20 years of age, it is believed that any treatment which will have a lasting and positive effect on site productivity will lessen the severity of the disease.

The study described here was installed primarily to test the value of subsoiling on a littleleaf site, but also to determine whether applications of pine bark, interplanting of black locust, or sowing lespedeza are feasible for improving the pine growth on the experimental area.

MATERIALS AND METHODS

This experiment was installed on a typical littleleaf site in Elbert County, Georgia, where low-quality mixed pines and hardwoods had been harvested within the previous 6 months. After harvesting, the site was root-raked and windrowed. Texture of the topsoil was sandy clay loam in two of three study blocks and sandy loam in the third; all soil was in the Madison series. Erosion had been extensive because of

poor agricultural practices in the past, and on many plots most of the original topsoil was gone.

A split-plot design with three replicate blocks was employed. Blocks measured 33.5 m × 143.2 m; half of each block was subsoiled (major plots). Subsoiled furrows were spaced 0.8 m apart and 0.6 to 0.8 m deep. Each block contained eight minor plots, 33.5 m × 33.5 m, with half of each plot in the subsoiled area. Four randomly selected plots in each block were planted with 1-0 loblolly pine and four with 1-0 shortleaf pine seedlings. Spacing was 3.0 m between rows and 1.5 m within rows. Minor treatments applied to each species were: (1) control (no treatment), (2) 8-cm-deep broadcast application of pine bark prior to subsoiling, (3) sowing of *Lespedeza sericea* (Thumb.) Benth. (67 kg/ha), and (4) interplanting with black locust (*Robinia pseudoacacia* L.). On plots where black locust was interplanted, pine spacing was increased to 3.0 m within rows and a locust seedling was planted between each pine seedling in each row. No interplanting was done between rows. Each minor plot contained 32 trees and each major plot contained 48 trees of each pine species.

Loblolly pine, shortleaf pine, and black locust seedlings were obtained from Georgia Forestry Commission nurseries and planted in early March. Many seedlings did not survive planting shock and a spring drought, so replanting was done in late April.

Fusiform rust incidence was recorded after 3 years. After 5 years, height, root-collar diameter, and survival were recorded. All data were subjected to an analysis of variance.

RESULTS AND DISCUSSION

In the 5 years after planting, growth of pines was not improved by bark applications, interplanting with black locust, or sowing *Lespedeza sericea*. The bark that was available for the study, even though it was several years old, contained a lot of undecomposed wood chips. For at least the first 2 years, growth of pines in bark-treated plots was retarded by competition of wood-decay microoganisms for soil nitrogen. Although the long-term effects of this treatment may be positive, no benefits can be detected at this time.

Although survival and first-year growth of black locust were excellent, the trees were attacked by locust borers, *Megacyllene robiniae*, which severely restricted subsequent growth. If attacks by *M. robiniae* do not kill the locust trees, the fixation of nitrogen by these trees may eventually improve growth of pines (Ike and Stone 1958). However, there has been no detectable improvement so far.

Also aimed at increasing soil nitrogen, the sowing of *Lespedeza sericea* did nothing to enhance growth of pines. It should be noted, however, that good stands of lespedeza were obtained and that they persisted for the length of the study with very little spread outside the plot boundaries. Competition with lespedeza did not inhibit pine growth, and the nitrogen it adds to the soil may eventually improve pine growth.

On even poorer sites or with tree species that are more nutrient demanding, locust and lespedeza might have improved tree growth. Major improvements, however, may require protecting black locust from borer attack, and liming to raise pH since acidity inhibits nodulation of legumes (Dart 1975).

Subsoiling was beneficial to both loblolly and shortleaf pines. With loblolly, subsoiling increased height growth by 3.5 percent and root-collar-diameter growth by 9.4 percent. The resulting increase in tree volume of 19.3 percent was statistically significant (table 1). Subsoiling increased height growth of shortleaf by 17 percent, root-collar-diameter growth by 15 percent, and tree volume by a significant 38 percent. (table 1).

Incidence of fusiform rust was high, and growth of infected loblolly seedlings was undoubtedly reduced. Most loblolly pine mortality was caused by fusiform rust. In addition to killing many trees, rust caused others to form multiple leaders and adopt a bushy growth habit. Such plants were listed as *Cronartium* bushes in table 1 and were considered dead in estimates of survival. Although they may live many years, these loblolly pines will never produce marketable products. In spite of the fusiform rust problem, however, the average volume ($\bar{X}$ tree volume × the number of surviving trees) in loblolly pine plots (221,207 cm^3) far exceeded that in shortleaf pine plots (75,475 cm^3). Fusiform rust incidence could not be related to any of the minor treatments. It appears, however, that rust incidence was increased by subsoiling. Loblolly mortality, most of which was probably caused by fusiform rust, was significantly higher on subsoiled plots and occurred mostly after 2 years. Treatments that increase loblolly pine growth often also increase susceptibility of trees to rust infection. Shortleaf pine, a species not affected by fusiform rust, on the other hand, survived significantly better on subsoiled plots.

Subsoiling prior to planting eases planting considerably, breaks any hardpan that may exist, and on many sites should greatly improve water penetration. Thus, although the intensity of subsoiling needed for a given site will undoubtedly vary, it appears that some subsoiling is desirable on many sites. The need for additional work on subsoiling techniques as they

relate to different soils is apparent and urgently needed to achieve more production of timber per unit area, particularly on devastated sites.

Table 1.—Effects of subsoiling on survival and growth of loblolly and shortleaf pine seedlings after 5 years

| Major plots treatments | Survival[1] after | | Height | Diameter | Volume[2] | Fusiform rust infection | |
	2 years	5 years				Cronartium bushes	Trees[3]
	Percent		cm	cm	cm³	Percent	
				LOBLOLLY			
Subsoiled	98.1 a	58.3 a	318 a	8.1 a	22,417 a	11.9 a	6.25 a
Not subsoiled	96.1 a	77.6 a	307 a	7.4 a	18,791 b	5.4 b	12.5 a
				SHORTLEAF			
Subsoiled	98.0 a	94.8 a	189 a	5.1 a	5,901 a	—	—
Not subsoiled	93.9 a	89.6 b	162 a	4.4 a	4,286 b	—	—

[1] *Cronartium* bushes are not included as survivors.

[2] Volume = (diameter)² × height.

[3] None of these trees are *Cronartium* bushes, but all became infected after planting.

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poor agricultural practices in the past, and on many plots most of the original topsoil was gone.

A split-plot design with three replicate blocks was employed. Blocks measured 33.5 m × 143.2 m; half of each block was subsoiled (major plots). Subsoiled furrows were spaced 0.8 m apart and 0.6 to 0.8 m deep. Each block contained eight minor plots, 33.5 m × 33.5 m, with half of each plot in the subsoiled area. Four randomly selected plots in each block were planted with 1-0 loblolly pine and four with 1-0 shortleaf pine seedlings. Spacing was 3.0 m between rows and 1.5 m within rows. Minor treatments applied to each species were: (1) control (no treatment), (2) 8-cm-deep broadcast application of pine bark prior to subsoiling, (3) sowing of *Lespedeza sericea* (Thumb.) Benth. (67 kg/ha), and (4) interplanting with black locust (*Robinia pseudoacacia* L.). On plots where black locust was interplanted, pine spacing was increased to 3.0 m within rows and a locust seedling was planted between each pine seedling in each row. No interplanting was done between rows. Each minor plot contained 32 trees and each major plot contained 48 trees of each pine species.

Loblolly pine, shortleaf pine, and black locust seedlings were obtained from Georgia Forestry Commission nurseries and planted in early March. Many seedlings did not survive planting shock and a spring drought, so replanting was done in late April.

Fusiform rust incidence was recorded after 3 years. After 5 years, height, root-collar diameter, and survival were recorded. All data were subjected to an analysis of variance.

RESULTS AND DISCUSSION

In the 5 years after planting, growth of pines was not improved by bark applications, interplanting with black locust, or sowing *Lespedeza sericea*. The bark that was available for the study, even though it was several years old, contained a lot of undecomposed wood chips. For at least the first 2 years, growth of pines in bark-treated plots was retarded by competition of wood-decay microorganisms for soil nitrogen. Although the long-term effects of this treatment may be positive, no benefits can be detected at this time.

Although survival and first-year growth of black locust were excellent, the trees were attacked by locust borers, *Megacyllene robiniae*, which severely restricted subsequent growth. If attacks by *M. robiniae* do not kill the locust trees, the fixation of nitrogen by these trees may eventually improve growth of pines (Ike and Stone 1958). However, there has been no detectable improvement so far.

Also aimed at increasing soil nitrogen, the sowing of *Lespedeza sericea* did nothing to enhance growth of pines. It should be noted, however, that good stands of lespedeza were obtained and that they persisted for the length of the study with very little spread outside the plot boundaries. Competition with lespedeza did not inhibit pine growth, and the nitrogen it adds to the soil may eventually improve pine growth.

On even poorer sites or with tree species that are more nutrient demanding, locust and lespedeza might have improved tree growth. Major improvements, however, may require protecting black locust from borer attack, and liming to raise pH since acidity inhibits nodulation of legumes (Dart 1975).

Subsoiling was beneficial to both loblolly and shortleaf pines. With loblolly, subsoiling increased height growth by 3.5 percent and root-collar-diameter growth by 9.4 percent. The resulting increase in tree volume of 19.3 percent was statistically significant (table 1). Subsoiling increased height growth of shortleaf by 17 percent, root-collar-diameter growth by 15 percent, and tree volume by a significant 38 percent. (table 1).

Incidence of fusiform rust was high, and growth of infected loblolly seedlings was undoubtedly reduced. Most loblolly pine mortality was caused by fusiform rust. In addition to killing many trees, rust caused others to form multiple leaders and adopt a bushy growth habit. Such plants were listed as *Cronartium* bushes in table 1 and were considered dead in estimates of survival. Although they may live many years, these loblolly pines will never produce marketable products. In spite of the fusiform rust problem, however, the average volume ($\bar{X}$ tree volume × the number of surviving trees) in loblolly pine plots (221,207 cm^3) far exceeded that in shortleaf pine plots (75,475 cm^3). Fusiform rust incidence could not be related to any of the minor treatments. It appears, however, that rust incidence was increased by subsoiling. Loblolly mortality, most of which was probably caused by fusiform rust, was significantly higher on subsoiled plots and occurred mostly after 2 years. Treatments that increase loblolly pine growth often also increase susceptibility of trees to rust infection. Shortleaf pine, a species not affected by fusiform rust, on the other hand, survived significantly better on subsoiled plots.

Subsoiling prior to planting eases planting considerably, breaks any hardpan that may exist, and on many sites should greatly improve water penetration. Thus, although the intensity of subsoiling needed for a given site will undoubtedly vary, it appears that some subsoiling is desirable on many sites. The need for additional work on subsoiling techniques as they

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Slit Application of Fertilizer Tablets and Sewage Sludge Improve Initial Growth of Loblolly Pine Seedlings in the Tennessee Copper Basin

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Forest starter fertilizer tablets (9 and 21 g) and dried sewage sludge (30, 60 and 90 g) were placed in slits in soil near recently planted loblolly pine seedlings in the Tennessee Copper Basin. Survival was not affected by treatment. However, by the end of the third year, the 21 and 9 g fertilizer tablets increased seedling volume by 20 and 9 fold, and the 90, 60 and 30 g sludge treatments increased volume by 8, 4 and 3 fold over control nonfertilized seedlings. The results show that slow-release fertilizer, either a commercially produced fertilizer tablet or dried sewage sludge, can be useful in stimulating seedling growth for the reclamation of nutrient deficient sites such as those in the Copper Basin of Tennessee.

THE COPPER BASIN in southeastern Tennessee became a mining and processing center for copper, iron, zinc, and other minerals soon after these metallic ores were discovered there in 1843. Mining activities contributed much to the economy of the region, but for many years they also decimated the environment. Valuable timber was indiscriminately cut for fuel to roast ore. "Heap-roasting" produced large quantities of sulfur dioxide gas at ground level which killed most vegetation over several thousand acres (Seigworth, 1943), and with the loss of vegetation, erosion of top soil became a serious problem.

Today, Cities Service Company, owner of the mines and processing facilities in the Basin, has a strong reforestation program and supports research to improve methods for rehabilitating the more eroded parts of the Basin. Previous to acquisition by the Cities Service Company in 1964, the Tennessee Copper Company (Seigworth, 1943) and the Tennessee Valley Authority (Allen, 1950) also had reforestation programs that were partially successful, particularly around the fringes of the basin.

Difficulties in reclaiming this area include the lack of adequate topsoil, a highly erodible subsoil of the Haysville sandy loam series, low quantities of available nutrients for tree growth, and

frequent heavy rainstorms which greatly increase soil erosion (Allen, 1950). Recent technological innovations in the production of sulfuric acid have greatly reduced sulfur dioxide emissions which previously reached concentrations that were injurious to plants.

Applications of dried sewage sludge have been shown to benefit forest sites of low fertility, such as drastically disturbed surface-mine spoils and eroded areas (Sopper, 1970; Berry, 1977). On routine sites, Gagnon (1974) increased growth of jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* Moench. Voss) by applying dry sewage sludge to established stands. Smith and Evans (1977) presented a review of literature relating to the disposal of wastes, including dried sewage sludge, on forest land. Recently Berry and Marx (1976) reported that sewage sludge mixed with a heavy clay forest soil of the Cecil-Madison series significantly increased growth of shortleaf and loblolly pine (*P. echinata* Mill. and *P. taeda* L.) seedlings. Berry and Marx (1977) also showed that growth of loblolly pine seedlings is greatly increased on kaolin spoil when amended with sewage sludge.

The value of dried sewage sludge broadcasted at high rates, therefore, has been demonstrated on certain disturbed sites. On disturbed areas that are remote from a source of sludge, how-

Table 1. Soil analyses* from the Copper Basin of Tennessee

		ppm						Total N	% Organic matter	Cation exchange capacity me/100 g
pH	Available P	Exchangeable								
		K	Ca	Mg	Mn	S				
4.4	1.2	12	2.9	2.0	3.2	448	300	0.83	3.3	

*Values are means of 3 samples. Analyses performed by C. G. Wells, USDA Forest Service, Forestry Sciences Laboratory, Research Triangle Park, NC 27709.

ever, the cost of hauling and distribution can be quite high. Slit applications of sludge to individual trees at planting would require far less sludge per hectare and may be a feasible alternative to broadcasting.

Slow release fertilizers in tablet form were developed in the late 1950's by Austin and Strand (1960) and have been used to increase growth of trees in the Northwest. White (1963) reported growth increases of white pine and white spruce by slit applications of several materials, including forest "starter" tablets. Meskimen (1971) in Florida also achieved a stimulation of early growth of *Eucalyptus camaldulensis* on sandy soil using fertilizer tablets.

Table 2. Plant Nutrients, Na, Al, and Organic Matter in Dried Sewage Sludge from Sewage Treatment Plants at Athens, Georgia*

Element	Acid extractable**	Total***
	ppm	
N	—	21,500
NO_3-N	3,613	—
P	161	9,034
K	41	4,034
Ca	580	13,750
Mg	147	284
Zn	205	1,105
B	1.14	17.5
Mn	59	133
Fe	101	7,608
Na	205	344
Cu	18	414
Al	134	5,945
Organic matter (%)		48.9

    * Analyses performed by the Soil Testing Laboratory, Cooperative Extension Service, University of Georgia, Athens 30602. Each value is the average of six analyses.
    **Double acid extraction method ($0.05N$ HCl in $0.025N$ H_2SO_4).
***Total nitrogen analyzed by Kjeldahl method: NO_3-N by steam distillation procedures: organic matter determined by ashing: and metallic elements dryashed and determined by flame-emission spectroscopy.

This paper reports an evaluation of the potential use of dried sewage sludge and forest starter fertilizer tablets placed in slits to stimulate growth of loblolly pine seedlings in the Tennessee Copper Basin.

MATERIALS AND METHODS

The study was installed on a severely eroded section of the Tennessee Copper Basin that was completely devoid of vegetation. Prior to installation of the study, the area was subsoiled using a large crawler-type tractor equipped with 2 "ripping" shanks, which produced furrows 2.4 meters apart and fractured the soil approximately 90 cm deep. Subsoiling appeared to increase water infiltration and greatly facilitated planting of seedlings.

Prior to planting, 3 soil samples (each a composite of 3 subsamples) were collected from the study area, air dried and crushed with a roller to pass through a 2-mm mesh sieve. Extractions for P, K, Ca, Mg, and Mn were made by shaking 10 g of soil in 50 ml of a solution of $0.05N$ $HCl+0.025N$ H_2SO_4 for 5 minutes. P was determined colorimetrically, and concentrations of cations were measured by atomic absorption. Total N was determined by Kjeldahl, organic matter by chromic acid wet digestion, cation exchange capacity (CEC) by saturation with NH_4^+ and replacement with K^+. Soil sulfur was extracted with $0.05N$ NH_4Ac (pH 7) and analyzed turbimetrically. Soil pH was measured by a glass electrode in a soil paste (C. G. Wells, personal communication). Results of the soil analyses are shown in Table 1.

Sewage sludge (Table 2) obtained from Athens, Georgia, sewage treatment plants was processed through a soil shredder which reduced particle size to approximately 1 cm³ or smaller. The sludge was ovendried at 95°C for 48 hrs. Individual volumes of sludge were preweighed in the laboratory and taken to the field in small polyethylene bags.

Table 3. Analyses of Agriform Forest Starter Tablets According to Manufacturer

Element	9 g tablet	21 g tablet
	%	%
Total Nitrogen	22.0	20.0
Water soluble nitrogen	6.6	7.0
Water insoluble nitrogen	15.4	13.0
Available Phosphorus Acid (P_2O_5)	8.0	10.0
Soluble Potash (K_2O)	2.0	5.0
Combined Calcium	3.0	2.6
Combined Sulfur	1.0	1.6
Elemental Iron	0.5	0.35

Loblolly pine seedlings were planted by hand with a planting bar or "dibble." In this method, a hole for the seedling is opened with the bar, the seedling is placed in the hole, and the hole is closed by rocking the bar in a second hole parallel to the first and 8 cm away. The fertilizer tablets or sludge were placed in these closing holes, which were approximately 8 cm deep. The closing hole was covered with loose soil.

The treatments were as follows: 1) Control (no treatment); 2) 30 g of sludge; 3) 60 g of sludge; 4) 90 g of sludge; 5) 9 g Agriform forest starter tablet (Sierra Chemical Co., Milpitas, CA); and 6) 21 g Agriform starter tablet (Table 3).

The study consisted of a completely randomized plot design with 5 replicate plots per treatment. Each plot contained 27 seedlings planted in a single row. Seedlings were planted in March 1975, 1.2 m apart in rows that were placed in the subsoiled furrows. The study was installed in a gently sloping area 35 m × 70 m. Two weeks after planting, initial height, diameter and survival data were taken to determine whether or not there were differences in seedling size among plots. Subsequent data have been taken yearly during dormant seasons. Data were analyzed by analysis of variance, and means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

During the first 3 growing seasons, seedlings on the control plots grew poorly (Fig. 1) while seedlings in all of the 5 treatments grew significantly better. The fertilizer tablets were most convenient to use and, in this study, most effective in stimulating seedling growth (Table 4). The ranking of treatments in effectiveness was evident after the first season and became even more pronounced after each succeeding growing sea-

son (Fig. 2). By the end of the third year, the 21 and 9 g fertilizer tablets increased seedling volume by 20 and 9 fold and the 90, 60 and 30 g sludge treatments increased volume by 8, 4 and 3 fold over the unfertilized control seedlings. Although fertilizer tablets stimulated the fastest early growth, the long-term effects on root development and mycorrhizal development need further evaluation. Marx et al. (1977) found that high soil fertility produced by inorganic fertilizers decreased sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by *Pisolithus tinctorius*. Berry and Marx (1977) found, however, that loblolly pine seedlings growing in kaolin spoil could readily tolerate sewage sludge amendments up to 138 metric tons per hectare without a significant reduction in percent ectomycorrhizae.

Most of the mortality, somewhat higher than expected, was related to washing into subsoiled furrows and poor seedling quality; after 3 years, differences in survival between treatments are not statistically significant. In subsequent studies involving similar materials, more time for settling of furrows has been allowed after subsoiling (1 month to 1 year) and survival has been excellent (80 to 100 percent).

Slit applications of nutrients give maximum benefit to tree seedlings and minimum stimulation to weeds. Weed competition is often a major cause of seedling mortality in plots amended with broadcasted sludge. Berry (1977) applied dried sewage sludge in broadcast applications to eroded forest lands at rates of 0, 17, 34, and 69 metric tons per hectare. Mortality of both loblolly and shortleaf pines increased with increased sludge levels. Although shortleaf pine had good survival on control plots, survival was only 22 percent on plots with 69 metric tons per hectare of sludge. Production of weed biomass was in-
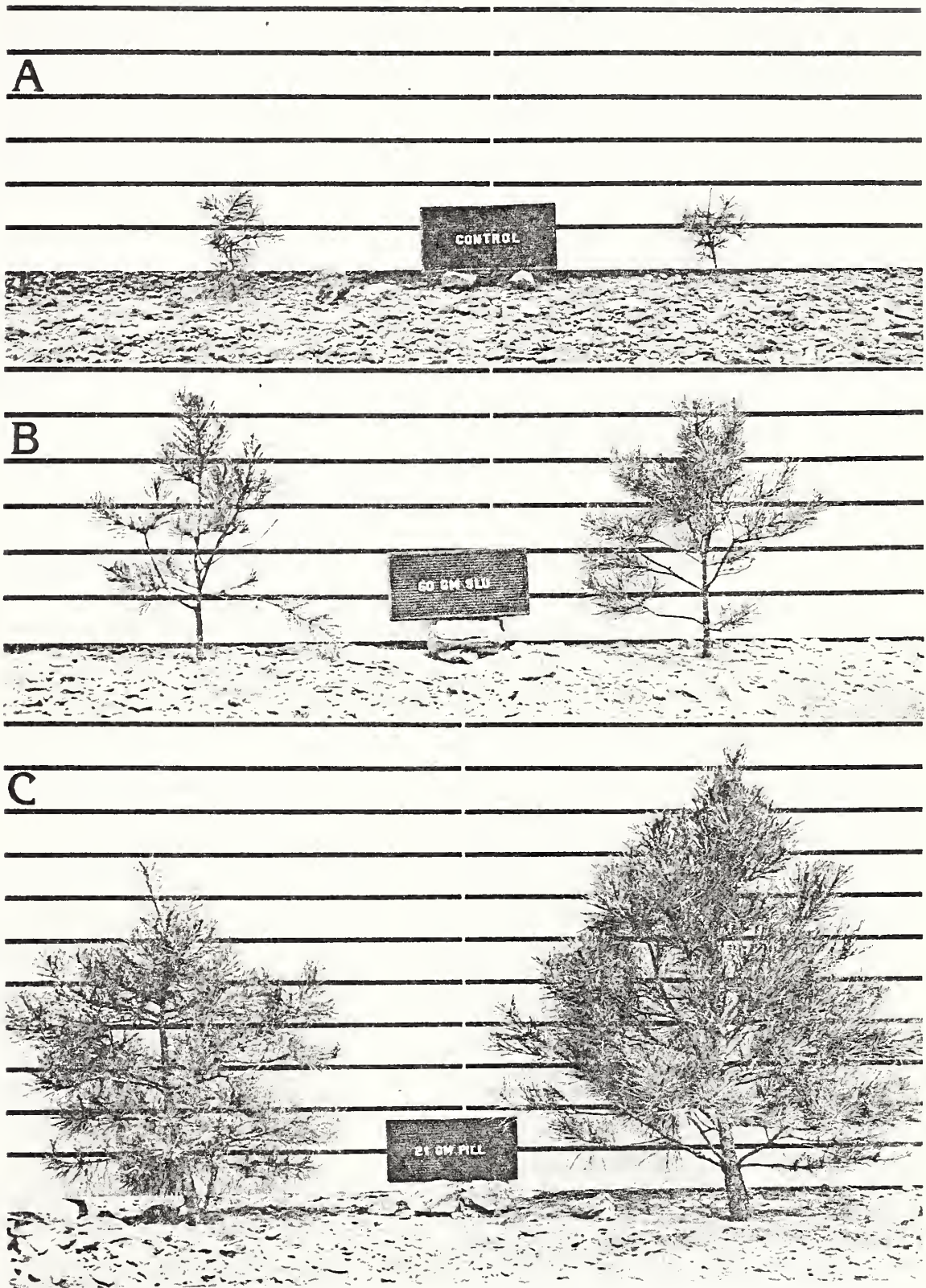
Figure 1. Effects of slit applications of 60 g of dried sewage sludge (B) and a 21-g forest starter tablet (C) on growth of loblolly pine seedlings. Control seedlings (no treatment) are shown at A. Photographed after third growing season.
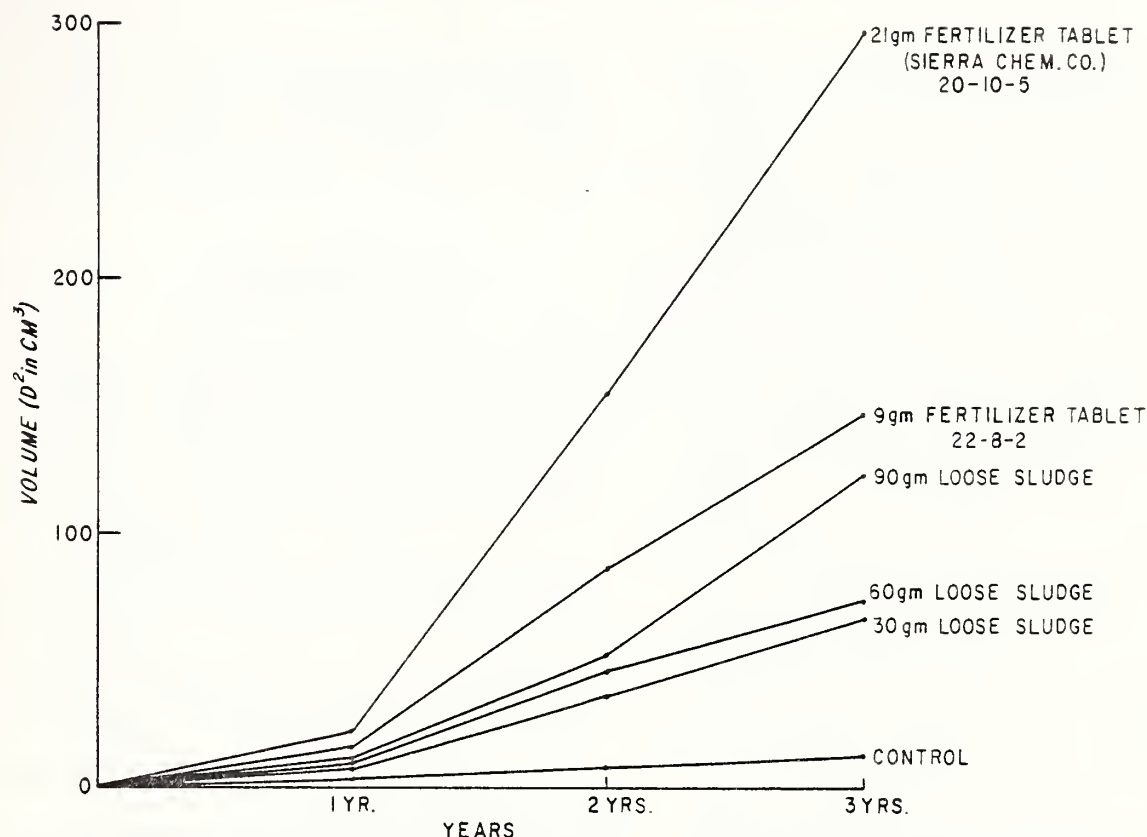
Figure 2. Effects of slit applications of dried sewage sludge and Agriform fertilizer tablets on early growth of loblolly pine seedlings at Copper Basin, Tennessee.

Table 4. *Effects of Slit Applications of Dried Sewage Sludge and Forest Starter Tablets* on Growth of Loblolly Pine Seedlings After 3 Growing Seasons*

Treatment	Survival percent	Stem height (cm)	Stem diameter (mm)	Volume** (cm^3)
21 g tablet	63 a	56.5 a	20.0 a	297.9 a
9 g tablet	69 a	49.4 a	15.6 b	147.9 b
90 g sludge	63 a	46.3 bc	15.0 b	124.6 bc
60 g sludge	70 a	41.4 c	12.2 c	74.0 c
30 g sludge	56 a	41.8 c	11.6 c	68.2 cd
Control	45 a	26.2 d	6.3 d	14.3 d

* Sierra Chemical Company, Milpitas, California.
** Diameter squared × height.
All treatments followed by same letter do not differ significantly at P=0.05.

creased nearly five fold by application of sludge on the same plots and was related to seedling mortality.

The risk of contaminating water supplies or food chains with heavy metals, nitrates or animal pathogens by slit application of sludge on dis-

turbed areas is not believed to be very high. Relatively little sludge would be used at any one location, and each individual tree application would be completely surrounded and covered by soil which has recently been recognized for its "filtering" qualities (Ellis, 1973: Miller, 1973). This does not imply that sludge from any source should be used indiscriminately on any site; the possibility of soil and water contamination must always be considered.

Two hundred ml of dried sludge weighs approximately 90 g, the highest rate used in this study. For routine practice in the field, this amount could be determined by volume measurement. Slit application of sewage sludge during reforestation of disturbed areas looks promising, because it stimulates growth of tree seedlings and because it is easier and cheaper than broadcast application.

The results of this study indicate that slit applications of nutrients, particularly forest starter tablets and small quantities of sewage sludge, are an alternative to broadcast applications. While broadcast applications of fertilizer or sewage

sludge probably will result in more biomass production per hectare, slit applications are less expensive, easier to apply on rugged terrain, and less stimulating to growth of competing vegetation.

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Significance of Various Soil Amendments to Borrow Pit Reclamation with Loblolly Pine and Fescue

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Loblolly pine seedlings with ectomycorrhizae formed by *Pisolithus tinctorius* (Pt) or naturally occurring *Thelephora terrestris* (Tt) were planted on a borrow pit in South Carolina in plots with no amendment; with fertilizer plus dolomitic limestone alone and with pine bark or bottom ash or bark and ash together; or with dried sewage sludge alone and with bark or ash or bark and ash together. All plots were subsoiled, disked, and seeded to fescue grass before planting pine seedlings. Naturally occurring Pt formed abundant ectomycorrhizae on all Tt seedlings by the end of the first season, precluding any specific ectomycorrhizal fungus effect for the duration of the study.

Sewage sludge alone or with bark or ash amendments dramatically improved pine seedling growth and grass biomass in comparison with other soil treatments. Mean seedling volume (D^2H) was 28 times greater and grass biomass was five times greater in the sludge plots than on nonsludge plots. Generally, soil amended with sludge contained more N, P, organic matter, and had a higher cation exchange capacity than soil of other treatments. Foliage of pine seedlings in sludge-amended soil also contained more N and less Ca than other seedlings. The significance of these results to reclamation of borrow pits is discussed.

INTRODUCTION

MANY CONSTRUCTION projects, such as dams, highways, and buildings require extensive earth fill to meet design criteria. When insufficient soil fill is available onsite it becomes necessary to "borrow" soil from another location. Generally, the resulting borrow pits are excavations from which all the A and B soil horizons have been removed. Compared with other disturbed areas such as surface mines, individual borrow pits may be relatively small in size, however, they do represent a significant amount of

surface area throughout the country. For example, the Department of Energy's Savannah River Plant (SRP) near Aiken, South Carolina, covers about 81,000 ha of which approximately 1% is composed of borrow pits.

Blauch (1978) discusses the problems of borrow pit reclamation. Prescriptions for effective reclamation of borrow pits from area to area will vary considerably due to differences in soil type, slope, desired vegetation, climatic conditions, and other factors. In the past, most borrow pit revegetation consisted of little more than a single application of fertilizer, perhaps some scarification of the surface soil, and sowing grass seed or planting tree seedlings. It is doubtful, however, that such a minimal effort ever resulted in satisfactory reclamation of a borrow pit. On the SRP located in the upper Coastal Plain of South Carolina, a great deal more effort appears to be needed because the exposed clay surfaces are highly compacted and eroded, impervious to root growth, and are extremely low in available water, fertility, and organic matter. In an earlier

Figure 1. Borrow pit as it appeared before installation of experiments. Trees are 22 years old, only 2.5 to 5 m in height, and growth is stagnant.

borrow pit study at the SRP, Granade (1976) found that grasses responded to N and P fertilization but not to K or lime. McGregor and Goebel (1968) planted slash and loblolly pine (*Pinus elliotti* Engelm. and *P. taeda* L.) seedlings on another borrow pit after subsoiling the test area to depths of 38 to 46 cm in furrows 2 m apart. They were able to improve growth of both pine species with broadcast applications of ammonium sulfate.

Berry (1979) obtained increased growth of loblolly and shortleaf pine (*P. echinata* Mill.) on an eroded low-quality site in the Georgia Piedmont by subsoiling to a depth of 60 cm. Subsoiling loosens severely compacted soil and improves aeration as well as water and root penetration, and is now used frequently on a variety of sites.

In microplot studies, Berry and Marx (1976) reported that dried sewage sludge stimulated growth of loblolly pine seedlings in C horizon material of a Cecil-Madison series. A specific ectomycorrhizal fungus, *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt), was also found to increase growth more than naturally occurring ectomycorrhizal fungi. Pt is ecologically adapted

to adverse soil conditions and improves growth of pines on several kinds of adverse sites such as coal spoils (Marx and Artman, 1979), kaolin spoils (Otrosina, 1977), and poor reforestation sites (Marx et al., 1977). In another microplot study, Berry and Marx (1977) found that sewage sludge also stimulated loblolly pine seedlings in kaolin spoil.

Another problem at the SRP is the disposal of bottom furnace ash which accumulates at a rate of approximately 45,455 metric tons per year. Horton and McMinn (1977) found that several pine species survived better when growing on bottom furnace coal ash in an ash basin with no soil than when planted in soil, and could find no evidence of toxic levels of minor elements by visual symptoms or by foliar chemical analyses.

Difficulties encountered in past attempts to revegetate borrow pits appear to have been caused by compact soil low in fertility and organic matter. Our aim in this study was to determine whether borrow pits could be reclaimed by deep subsoiling, applying various waste products, and by planting pine seedlings colonized with a specific ectomycorrhizal fungus, Pt.

MATERIALS AND METHODS

Seedling Production

Loblolly pine seedlings (seed from South Carolina Piedmont source) were grown for 10 months in fumigated soil either infested or noninfested with vegetative inoculum of Pt (isolate 138). Procedures for producing seedlings have been discussed (Marx and Artman, 1979). Seedlings were lifted in early spring of 1976, graded to a root-collar diameter between 3.0 and 4.5 mm and a height between 16 and 19 cm. Approximately 70% ($\pm$15%) of the feeder roots of all seedlings were ectomycorrhizal. Seedlings that had been inoculated with Pt had approximately two-thirds of their ectomycorrhizae formed by that fungus. Most of the ectomycorrhizae on seedlings from noninfested soil were formed by *Thelephora terrestris* [Ehrh. ex Fr.] (Tt). After lifting and grading, all seedlings were stored at 5°C in plastic-lined, kraft paper bags in moist peat moss for 1 week before planting.

Site Preparation

The borrow pit site, composed of Fuquay and Wagram soils, was created in 1950–1952, and in 1953 reclamation was attempted by machine planting with loblolly pine seedlings. In 1976, surviving trees were severely stunted and yellow, and with roots barely under the soil surface (Fig. 1). Many surviving trees were windthrown, but those still standing were only 2.5 to 5 m tall. Many standing trees had such limited root development that they could readily be pulled from the soil by hand. Although a thin layer of litter was present under some trees, no understory grass or shrubs were growing to retard rapid surface runoff. Basidiocarps of Pt were numerous and the mustard-yellow ectomycorrhizae were abundant on pine roots.

Preparation for this study was initiated in the summer of 1975 by removing all trees and large roots and subsoiling the entire site by ripping furrows 0.9 m deep, spaced 1.2 m apart, in both north-south and east-west directions. The site was double disked to break large clods of clay. Plots measuring 8.5 × 8.5 m were separated by aisles 3 m wide in one direction and 6 m wide in

the other. Amendments were broadcast by hand and incorporated by double disking. The two ectomycorrhizal treatments were combined with each of the following nine fertility treatments:

No treatment (control)
Fertilizer* and lime†
Fertilizer and lime + tree bark‡
Fertilizer and lime + ash‡
Fertilizer and lime + bark + ash
Sewage sludge
Sewage sludge + bark
Sewage sludge + ash
Sewage sludge + bark + ash

Anaerobically digested, dried sewage sludge obtained from treatment plants in Athens, Georgia, was found to contain about 2% N, 1% P, 0.5% K, and 50% organic matter (Berry and Marx, 1977). Milled pine bark (particle size 0.5 to 1.0 cm), shown to be an excellent medium for growth of pine seedlings (Rowan, 1978) and for other plant species (Brown and Pokorny, 1975), was purchased from a local supplier.

Bottom furnace ash, obtained from an ash basin on SRP, previously had been analyzed and found to contain at least 24 chemical elements which included 300 ppm P, 10,000 ppm K, and 10,000 ppm Ca (Horton and McMinn, 1977).

The entire borrow pit was seeded in the fall of 1975 with Ky 31 fescue (*Festuca arundinacea* Schreb.) at 34 kg/ha. Pine seedlings were planted in the spring of 1976 within 1 week after lifting from the experimental nursery. Twenty-five test seedlings, five rows of five seedlings each, with a spacing of 1.2 × 1.2 m were planted in each plot. In addition, border seedlings were planted on all sides of the plot.

In the fall of 1976, 1977, and 1978, growth data and samples of biomass, soil, and foliage were collected, and all plots were examined for fruit bodies of ectomycorrhizal fungi. However, since no unusual trends were discovered, only 1978 data are presented. Grass biomass from three randomly placed 0.33-m² subplots per plot was clipped, ovendried at 70°C for 96 h and weighed. After the first growing season, a root sample of each of five randomly selected seedlings per plot was carefully detached and examined for ectomycorrhizal development.

Soil samples were taken from each plot at a depth of 0 to 10 cm, air-dried at room temperature for 10 days, and chemically analyzed after double acid extraction with 0.05N HCl + 0.025N H_2SO_4. P was determined colorimetrically and cations by atomic absorption. Total N was determined by Kjeldahl, organic matter by wet oxi-

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*560 kg/ha of commercial 10-10-10 fertilizer.
†2,240 kg/ha of dolomitic limestone.
‡Sewage sludge, milled pine bark, and bottom furnace ash were applied at a rate of 125 m³/ha, or approximately 1.3-cm deep. With sewage sludge, this rate was equivalent to a dry weight of 34,000 kg/ha.

Table 1. Mean Growth and Survival of Loblolly Pine Seedlings and Grass Biomass Production after 3 Years on a Subsoiled Borrow Pit as Influenced by Different Soil Amendments*

Amendments†	Survival	Height	Root-collar diameter	Seedling volume	Grass biomass	Fusiform rust infection‡
	%	m	cm	cm³ (×10²)	g/m²	%
Control	81 a	0.63 c	1.9 b	4 c	0	15.9 de
Fertilizer & lime	77 a	0.72 c	2.0 b	4 c	29 c	16.2 cde
Bark + fertilizer & lime	79 a	0.57 c	1.6 b	2 c	86 c	16.4 cde
Ash + fertilizer & lime	80 a	0.73 c	1.9 b	5 c	31 c	20.8 b
Bark + ash + fertilizer & lime	86 a	0.59 c	1.6 b	3 c	98 c	12.7 e
Sewage sludge	74 a	2.23 ab	6.4 a	100 ab	353 a	19.7 bc
Bark + sewage sludge	77 a	2.13 b	6.0 a	85 b	243 a	20.7 b
Ash + sewage sludge	72 a	2.30 a	6.2 a	104 a	411 a	17.5 bcd
Bark + ash + sewage sludge	75 a	2.37 a	6.3 a	107 a	342 a	28.5 a

*Means in a column followed by the same letter are not significantly different ($p = 0.05$).

†Fertilizer and lime: 560 kg/ha of 10-10-10 + 2,240 kg/ha of dolomitic limestone. Bark, bottom ash, and sewage sludge broadcast evenly on the soil surface to a depth equal to 1.25 cm per each material. All plots double disked to incorporate amendments.

‡Percent of trees that became infected after planting.

Figure 2. Trees on a control plot (foreground) are contrasted with trees on sludge plots. Exposed wall in background indicates amount of earth removed from pit.

Table 2. Chemical Soil Properties in a Borrow Pit 3 Years after Addition of Amendments*

Amendment	ppm					Organic matter %	CEC me/100 g	pH
	N	P	K	Ca	Mg			
Control	112 d	7 b	6 d	4 d	11 c	0.4 c	1.4 c	4.2 bc
Fertilizer & lime	153 cd	13 b	7 d	16 c	65 a	0.6 c	1.7 c	4.9 a
Fertilizer & lime + bark	160 cd	7 b	11 bc	19 bc	76 a	1.6 b	—	4.6 ab
Fertilizer & lime + ash	177 cd	11 b	18 a	19 bc	79 a	0.6 c	—	4.9 a
Fertilizer & lime + bark + ash	291 c	16 b	18 a	21 bc	72 a	1.6 b	—	4.5 abc
Sewage sludge	595 b	84 a	7 d	22 abc	18 bc	1.6 b	2.0 bc	4.2 bc
Sewage sludge + bark	597 b	72 a	8 cd	22 abc	17 bc	2.3 ab	2.6 b	4.0 c
Sewage sludge + ash	687 ab	93 a	11 bc	23 ab	17 bc	2.0 ab	2.6 b	4.4 abc
Sewage sludge + bark + ash	762 a	98 a	14 b	27 a	33 b	3.0 a	4.0 a	4.4 abc

*Values followed by the same letter within a column are not significantly different ($p = 0.05$).
Values are means of five samples which, for elements, represent extractable fractions.

dation chromic acid digestion, CEC by saturation with NH_4^+ and replacement with K^+, and soil pH by glass electrode in a soil paste.

Samples of current-year needles, removed from each plot tree, were combined into a plot sample and dried at 85°C for 72 h prior to tissue analysis. Total N was determined by Kjeldahl, and other elements by dry ash methods (Wells et al., 1973).

Data were analyzed by analysis of variance for a random block design, and treatment means were separated by Duncan's Multiple Range Test.

RESULTS

Mechanical analysis revealed the borrow pit to be of uniform texture, and consisting of a sandy-clay loam (21% clay, 75% sand, and 4% silt). Root evaluations at the end of the first growing season revealed that indigenous Pt had formed ectomycorrhizae on all trees regardless of their original ectomycorrhizal condition. This contamination probably was the result of residual inoculum remaining in the soil and small roots from the previous stand of pine. As a result of the loss of the ectomycorrhizal treatment variable, the experiment was analyzed only for effects of the soil amendments.

Fruit bodies of Pt and Tt were found in abundance in nearly all amended plots after the first season. The occurrence of Pt fruit bodies was not related to initial ectomycorrhizal condition of the test pine seedlings. Fruit bodies of Pt and Tt as well as those of *Rhizopogon roseolus* (Corda in Sturm) Th. M. Fr., *Suillus luteus* (L. ex Fr.) S. F. Gray, and *Laccaria laccata* (Scop. ex Fr.) Berk and Br. were observed during the following years, with more being produced in sludge-amended plots than in other plots. Few fruit bodies of any fungi were observed in non-amended (control) plots.

Seedlings grown on plots treated with sewage sludge alone or in combination with other amendments were significantly larger in height, diameter and volume (D^2H) than seedlings grown on plots without sewage sludge (Table 1, Fig. 2). The mean D^2H of seedlings grown on sludge-amended plots was approximately 20 times greater than that of seedlings grown on sludge-free plots. The addition of bark or ash to soil, either with or without sewage sludge, did not significantly affect growth of trees.

Plots that had been amended with sewage sludge had more soil nitrogen and phosphorus than plots amended with fertilizer and lime, while plots with bark and ash had more potassium (Table 2). Organic matter was greatest on sludge-amended plots or on fertilizer plots that had received bark. Analyses for cation exchange capacity (CEC) revealed that the addition of bottom ash or bark tended to increase CEC com-

Table 3. Foliar Analyses of Loblolly Pine Seedlings After 3 Years on a Borrow Pit*

Treatment	%					ppm					
	N	P	K	Ca	Mg	Mn	Fe	Na	Zn	Cu	Al
Control	0.91 e	.09 d	.31 c	.27 a	.14 a	442 a	78 a	161 a	34 d	1.3 bc	910 a
Fertilizer & lime	1.03 e	.10 c	.36 bc	.28 a	.15 a	154 c	69 ab	136 b	31 d	1.0 c	824 b
Fertilizer & lime + bark	1.16 cd	.12 abc	.42 ab	.29 a	.15 a	251 b	70 ab	124 ab	58 ab	1.8 abc	794 b
Fertilizer & lime + ash	1.08 de	.11 bc	.46 a	.27 a	.13 ab	183 bc	64 ab	117 ab	39 cd	1.5 abc	729 bc
Fertilizer & lime + bark + ash	1.10 de	.12 abc	.47 a	.30 a	.15 a	196 bc	67 ab	105 c	40 bcd	1.6 abc	757 bc
X̄†	1.09	.11	.43	.28	.14	196	67	120	42	1.5	776
Sewage sludge	1.25 bc	.12 abc	.29 c	.17 b	.09 bc	198 bc	47 b	125 ab	73 a	1.9 ab	613 d
Sewage sludge + bark	1.26 bc	.12 abc	.30 c	.15 b	.07 c	212 bc	41 b	128 ab	61 a	2.1 ab	631 d
Sewage sludge + ash	1.28 b	.12 abc	.32 c	.15 b	.06 c	168 c	46 b	130 ab	61 a	2.2 a	699 cd
Sewage sludge + ash + bark	1.40 a	.13 a	.35 bc	.17 b	.08 c	189 bc	43 b	125 ab	56 abc	2.3 a	749 bc
X̄‡	1.30	.12	.31	.16	.07	192	44	127	63	2.1	673

*Values followed by the same letter in a column are not significantly different ($p=0.05$).
†Means for all fertilizer and lime treatments.
‡Means for all sewage sludge treatments.

pared to plots with sewage sludge only, and the addition of both bark and ash to sewage sludge plots doubled the CEC compared to plots with sewage sludge alone (Table 2).

Many of the soil trends were also reflected in foliar analyses. For example, higher N and P values were found in foliage from plots with sewage sludge (Table 3). Foliage concentrations for all treatments with sewage sludge were above the generally accepted levels of 0.10% P (Wells et al., 1973) and 1.2% N.* Concentrations of some elements such as Zn and Cu, considered normal constituents of sewage sludge, were also highest on sewage sludge plots (Table 3). Aluminum, an element which may be toxic at high levels and a principle ingredient of clay, was highest in trees on control plots (Table 3).

Growth of trees was the same on plots that received fertilizer and lime as it was on plots that received no amendment. There was no measurable grass biomass produced on plots that received no amendment, while on plots receiving fertilizer and lime, grass production was approximately 60 g/m² and plots receiving sewage sludge produced over 300 g/m² during the third growing season. The amount of grass biomass

*Wells, C. G. U.S. Dep. of Agric. Forest Service, Forestry Sciences Laboratory, Research Triangle Park, N. Car. Personal communication—April 1980.

produced on sewage sludge plots has varied considerably from year to year. During the first growing season 372 g/m² of grass were produced, during the second growing season 465 g/m² were produced, and during the third season 337 g/m² were produced. The decrease during the third year is attributed to competition for moisture, nutrients, and sunlight by the pines.

Tree growth and the number of trees with field infections of fusiform rust were greatest on plots where sludge had been applied. Interestingly, however, trees on plots with the highest CEC, i.e., those receiving sewage sludge with bark and ash had a significantly higher rate of rust infection than trees on plots with sewage sludge alone or sewage sludge and ash, while mean tree volumes for these three treatments did not vary more than ±4%, and were not statistically different.

DISCUSSION

The addition of a 1.3-cm layer of dried sewage sludge to a disturbed site such as a borrow pit will furnish sufficient nutrients for reclamation with loblolly pine and grass. Improvement in soil physical properties is also expected, though not measured in this study. Although the study was only three years old when final data were taken, trees on the sludge plots are growing rapidly and the grass ground cover, which was established

Figure 3. Borrow pit 3 years after installation of experiment.

quickly, had checked soil erosion (Fig. 3). The amount of sludge applied in this study supplied 692 kg/ha of N, 346 kg/ha of P, and about 17,000 kg/ha of organic matter. This rate of nutrient application is considerably more than is regarded as necessary for reclamation of kaolin mining spoils (May, 1977). In addition, since many of the essential nutrients in sludge are in organic form, they are released slowly over a long period of time. The benefits of sludge treatment are expected to last until new soil formation can support tree growth.

A microbiological examination* of these plots revealed that several species of saprophytic soil bacteria and fungi are present in large numbers on sludge plots but are scarce or absent on the nonsludge plots. This is an indication of the soil-building potential of sewage sludge.

At the time the study was begun, bark also was regarded as a waste product. Today, many forest industries are burning all wood waste for fuel so the availability of bark for future reclamation projects may be extremely limited. The

use of bottom ash as a soil amendment, however, is a possibility worthy of further consideration since its production could reach 70 million tons annually by 1980 (Faber, 1976). Although ash did not appear to promote growth of trees or grass, it apparently had no deleterious effect, therefore a great deal could be disposed of on borrow pits prior to reclamation. Plots receiving sewage sludge with bark and ash had significantly more N and a significantly higher CEC than plots receiving sewage sludge and bark, or sewage sludge and ash. The long-term effect of bottom furnace ash in borrow pit reclamation needs further study.

While deep subsoiling was not a variable in this study, the study site was intensively subsoiled prior to application of amendments, it is regarded by the authors as a beneficial prerequisite to successful reclamation of many sites. Although subsoiling is often recommended as a means of improving forest sites, only a few reports (Berry, 1979) have presented evidence that tree seedlings exhibit a positive growth response to subsoiling.

Unfortunately, benefits of using bare-root planting stock that had been inoculated with Pt were precluded by the presence of indigenous

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*Wojcik, V. H. and D. H. Marx. Unpublished data. Southeast. For. Exp. Stn., Forestry Sciences Laboratory, Athens, GA.

inoculum of Pt remaining in the soil after site preparation. In another experiment on this same site, however, Ruehle (1980) found that containerized loblolly pine seedlings tailored with Pt ectomycorrhizae grew significantly better on sludge-amended borrow pit plots than seedlings colonized with Tt or noninoculated seedlings. Since Ruehle's trees were planted in the fall of 1976 rather than spring of 1976, it is likely that he encountered a lower level of indigenous Pt inoculum in the soil and therefore was able to detect a stimulation of growth by a Pt treatment.

The Savannah River Plant is located in an area of high fusiform rust hazard (Phelps, 1973). The incidence of field infections appeared to be related to treatment: Trees exhibiting the best growth appeared to be the most susceptible to the disease. This may be partially explained on the basis of "target" size, where the fastest growing trees also present the biggest target for infection (Rowan and Steinbeck, 1977). Trees on plots with the highest CEC, i.e., those receiving sludge with ash and bark, were more susceptible to rust infection than trees on any other plots receiving sludge. The high nitrogen concentration in the foliage offers a possible explanation for the higher rust susceptibility in this treatment.

This research has demonstrated that amelioration of a borrow pit, unsuited for growth of trees and grass, can be accomplished quickly by deep subsoiling and an application of a 1.3-cm layer of dried sewage sludge. The disposal of a 1.3-cm layer of bottom furnace ash on the site concurrent with sludge application did no harm and may eventually prove to be beneficial.

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DEELY (handwritten)

Increasing Endomycorrhizal Fungus Inoculum in Forest Nursery Soil With Cover Crops

Paul P. Kormanik, W. Craig Bryan, and Richard C. Schultz

ABSTRACT. *Corn, millet, sudex, and sorghum were all effective cover crops for increasing inoculum density of vesicular-arbuscular fungi (Glomus spp.) in nursery soils. Spore production was increased approximately 7 to 12 times, depending on the cover crop used. Sweetgum seedlings did not differ significantly in size on plots previously planted with any of the four cover crops. Eighty-nine percent of the sweetgum seedlings grown after cover cropping had root-collar diameters exceeding the minimum (0.25 inch) recommended for outplanting of this species.*

A reliable supply of high-quality seedlings is a prerequisite for successful artificial hardwood regeneration. Unfortunately, forest tree nurseries have not consistently produced seedlings of the commercially important hardwood species that form vesicular-arbuscular (VA) mycorrhizae. Development of such mycorrhizae early in the growing season is beneficial to seedling development and can result in consistent production of high-quality seedlings (Bryan and Kormanik 1977, Kormanik *et al.* 1976, South 1977). One method of increasing nursery soil inoculum levels is to plant cover crops having fibrous roots that are hosts for VA mycorrhizal fungi. Of course, crops which are preferred hosts for commonly occurring pathogenic fungi in nursery soils should be avoided.

Periodic fumigation is necessary in most nurseries; however, it simultaneously eliminates or reduces beneficial mycorrhizal fungi, as well as the root pathogens and weed seeds which are the targets of fumigation. It takes at least one growing season after fumigation to build up the inoculum potential of the VA fungi to effective levels. In an extensive study of various forest tree nurseries in the southeastern United States, Barnard (1977) reported that nurseries employing soil fumigation were characterized by low VA inoculum densities.

The purpose of this study was to determine the capacity of some common cover crops for increasing VA inoculum density in nursery soils and to determine how inoculum densities might affect the subsequent crop of hardwood seedlings.

METHODS

The study was established in 1973 at the Whitehall Experimental Forest maintained by the Forest Service in cooperation with the University of Georgia in Athens. Two experimental nursery beds (4 × 20 feet) were filled with a fumigated soil mixture consisting of a loamy forest topsoil, sand, and finely ground pine bark at a 1:1:1 ratio. These beds were inoculated with chopped sorghum roots obtained from greenhouse cultures known to contain a mixture of VA fungi (*Glomus* spp.). Two similarly constructed beds were filled with identical soil but were not inoculated. For two years, either sycamore (*Platanus occidentalis* L.) or sweetgum (*Liquidambar styraciflua* L.) seedlings were produced in these beds for various experimental purposes. In 1975, all seedlings were lifted and soil from the two inoculated beds was used to infest the two nursery beds which had remained nonmycorrhizal for two growing seasons. Sweetgum was then planted in all four beds.

Sweetgum seedlings were lifted in January 1976, and in April the four beds were assayed for endomycorrhizal spore density. Spores were

extracted from the soil with a flotation apparatus for nematode extraction described by Oostenbrink (1960). The spores retrieved on a 45-micron sieve were further separated from soil and root debris by the centrifugal-flotation technique (Jenkins 1964). Spore count, which is indicative of the endomycorrhizal inoculum density, showed that the beds contained approximately 50 spores/100 cm^3 of soil. This count is much lower than the mean of 14 spores/gram of soil reported to occur in eight recently fumigated southeastern nurseries (Barnard 1977).

In 1976, each of the four beds was divided into four equal compartments (4 × 5 feet) by inserting three steel plates across the width of each bed to a depth of approximately 24 inches. In early May, each compartment was sown with either corn, millet, sudex, or sorghum. All cover crops were fertilized and maintained under identical conditions. They were given an initial application of 10-10-10 fertilizer equivalent to 250 lb/acre. In addition they received three applications of ammonium nitrate (NH_4NO_3) during the growing season; equivalent rate for these three applications was 1,500 lb/acre. During the second year (1977), the same fertility regime was maintained for seedling production except that the NH_4NO_3 was applied every 10 days with appropriate reductions in the rate at each application.

Spore counts were made according to the previously described method at the end of the first growing season. Cover crops were turned under, and the beds lay fallow throughout the winter. In May 1977 half of each bed was sown with seed from one of two sweetgum mother trees. Seedling density was maintained at approximately six/ft^2. Height and root-collar diameters were measured on all seedlings at the end of the growing season. The study was analyzed as a split plot with nursery beds as the whole plot; cover crops and mother trees were the subplots.

RESULTS

All four cover crops induced appreciable numbers of *Glomus* spp. spores, although sorghum, with its more fibrous root system, induced a higher mean density of spores than any other crop (Table 1). Spore production was increased approximately 7 to 12 times, depending on the cover crop used. However, no correlation was observed between spore production and whether the beds had been the source of inoculum or had previously been in the nonmycorrhizal test sequence.

Although statistical differences were observed in both height and root-collar diameter among sweetgum seedlings grown in compartments planted with the different cover crops, these differences

Table 1. Spores extracted per 100 cm^3 of soil at the end of the growing season.[1]

Bed no.	Corn	Millet	Sudex	Sorghum
1	603	568	362	906
2	360	474	342	669
3	209	284	535	305
4	214	509	675	603
Mean	346	459	479	621

[1] *Initially, the soil contained 50 spores per 100 cm^3. Variation among replicates of different crop types eliminated statistical significance of different spore levels.*

were of little practical significance. Mean height of sweetgum seedlings from the two mother trees in the various treatments varied from 25 to 27 inches. Mean root-collar diameters varied from 0.28 to 0.31 inch. The seedlings were of uniform quality with a coefficient of variation for total height of only 13 percent and for root-collar diameter of 16 percent. Of the 1,440 seedlings in the test, 89 percent had root-collar diameters greater than 0.25 inch, the minimum size recommended for successful outplanting of this species (Belanger and McAlpine 1975).

DISCUSSION

Any of the cover crops in this test could be used to increase the inoculum potential of endomycorrhizal fungi in nurseries by at least 7 times. Because we have tested these crops on a *Glomus* mixture, we cannot speculate on their ability to work with a specific root symbiont or another mixture that might be present in a given forest nursery. There is little doubt, however, that these plants will be effective with many species of endomycorrhizal fungi; they are frequently used as nursery crops to increase inoculum for research purposes.

Corn was the least desirable of the four cover crops tested. Spore production on this crop, while not statistically different from the other crops, was numerically the lowest, probably because corn has a coarser, less fibrous and dense root system. Inoculum density not only includes spore production but is affected by infested roots that remain after the cover crop has matured. Since the other three cover crops leave a heavier root mass in the soil, their inoculum densities are greater than that of corn. Sudex, millet, and sorghum also mature much later in the season than corn, yielding a longer period for effective root growth and concomitant spore production.

Spore production was apparently adequate with all cover crops to assure endomycorrhizal development early in the season. Although sweetgum seed were sown almost 2 months later

than desirable for the Athens area and had an effective growing season of only 18 weeks, 89 percent of the seedlings had root-collar diameters exceeding the minimum (0.25 inch) recommended for outplanting. If the growing season had been 26 weeks, undoubtedly more than 89 percent of the seedlings would have exceeded the 0.375-inch root-collar diameter preferred by many foresters for sweetgum planting stock.

It must be emphasized that the use of any cover crop after fumigation must be accompanied by careful monitoring of destructive root pathogens that may occur in different nursery soils. In nurseries with soil-borne disease problems, even good fumigation will probably leave viable inocula of pathogenic fungi. It would be preferable to determine which cover crop is the least desirable host for root pathogens in a specific nursery.

Soil fumigation must precede the sowing of the cover crop, since fumigation after cover cropping will eliminate or reduce the endomycorrhizal fungus inocula. Thus, if a cover crop is planted, fumigation for weed control must be replaced by the use of herbicides. Unfortunately, little is currently known about the effects of specific herbicides on endomycorrhizal inocula. Continued monitoring should also accompany successive tree rotations to detect buildup of root pathogens.

If cover cropping is adopted in hardwood nurseries, more research will be needed on how soil types, fertilizer regimes, and herbicides affect the density of endomycorrhizal inoculum, production of quality seedlings, and pathogen populations. This need for additional work should not discourage nurserymen from implementing the best management practices currently available to improve production of important species [i.e., sweetgum, green ash (*Fraxinus pennsylvanica* Marsh.), sycamore, yellow-poplar (*Liriodendron tulipifera* L.) and walnut (*Juglans nigra* L.)].

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Influence of Endomycorrhizae on Growth of Sweetgum Seedlings From Eight Mother Trees

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ABSTRACT. Sweetgum seedlings from eight mother trees were grown in fumigated soil with or without the endomycorrhizal fungus *Glomus mosseae* at four levels of soil fertility for one growing season in nursery microplots. Nonmycorrhizal seedlings of all families died or failed to exceed 5 cm in height regardless of soil fertility. Endomycorrhizal seedlings suffered little mortality, averaged about 36 cm in height, and fertility level did not significantly influence their biomass. These results demonstrate that to increase the percentage of plantable seedlings in nurseries sweetgum seedlings must be endomycorrhizal. The data further suggests that adequate endomycorrhizal inoculum in nursery beds can allow the use of less fertilizer than has been customary for production of sweetgum seedlings. FOREST SCI. 23:500–505.

ADDITIONAL KEY WORDS. *Liquidambar styraciflua, Glomus mosseae*, fertilizer.

SWEETGUM (*Liquidambar styraciflua* L.) is a commercially important hardwood species in the southern United States where it competes successfully in natural stands with many other hardwoods and with southern pines. However, attempts to artificially regenerate sweetgum have often failed because of slow height growth for several years after planting. This slow erratic growth begins in the nursery where in spite of high fertility levels, it is difficult to regulate seedling quality (Webb 1969).

Growth of sweetgum seedlings can be enhanced with endomycorrhizae (Bryan and Ruehle 1976, Bryan and Kormanik 1977). However, endomycorrhizal inoculum may be limiting in forest nursery soils during a considerable portion of the growing season because of fumigation and other cultural practices considered essential to control weeds and destructive root pathogens. Poor early season development of sweetgum seedlings may be related to a lack of endomycorrhizal inoculum in the zone of effective fumigation.

Many plants whose growth is enhanced by endomycorrhizae make acceptable growth if higher rates of soil fertility are maintained when endomycorrhizae are absent (Gerdemann 1968, Smith 1974). This interaction of soil fertility and endomycorrhizae has not been clarified for sweetgum seedling development. The purpose of this study was to determine whether different soil fertility rates could enhance growth of mycorrhizal sweetgum seedlings and to determine if artificial inoculation with the endomycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) (Gerd. and Trappe) of soils at various fertility levels would increase the size of seedlings produced by eight sweetgum families.

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METHODS

Seeds were collected from eight trees growing on the Scull Shoals Experimental Forest in Northeast Georgia. Five of the trees were from fertile bottomland sites and three from dry upland sites. The seeds were stratified in water at 2°–4°C for 24 days prior to sowing.

The study was carried out in 32 microplots in an experimental nursery at Athens, Georgia. The microplots were about 1 meter square × 0.3 meter deep and were constructed of redwood. The empty microplots were positioned on recently tilled soil and fumigated under plastic with methyl bromide (Dowfume MC-2, Dow Chemical Co.). The plastic was removed after 48 hours. The microplots were then filled with a similarly fumigated soil mixture consisting of equal volumes of sandy loam forest soil, sand, and finely ground pine bark. Analysis of this mixture revealed the following amounts of extractable ions in kg/ha: NO_3-N, 39.2; P, 25.8; K, 77.3 and Ca, 366.2. Hydrated lime (CaO) was added to the mixture in all microplots to bring elemental calcium up to 1120 kg/ha.

All microplots were inoculated with 270 grams of coarsely chopped sorghum roots from either pure pot cultures of sorghum containing *Glomus mosseae* or non-infected sorghum control pots. The top 3–10 cm of soil were removed and the inoculum was spread uniformly over the surface and the soil replaced. Root-washings from the pot cultures were passed through a 45 mesh sieve (openings smaller than the spore diameter of *G. mosseae*) and filtered through Whatman No. 1 paper to standardize the rhizosphere microflora at the time of sowing. The *G. mosseae* microplots received washings from noninfected sorghum pot cultures and the control microplots received washings from the sorghum pot cultures of *G. mosseae*.

Microplots were randomly assigned to four fertilizer treatments—140, 280, 560, and 1120 kg/ha of 10-10-10 fertilizer. These were incorporated into the top 7 to 10 cm of soil when the soil was removed for placement of inoculum. In addition, all microplots received 560 kg/ha of NH_4NO_3 three times during the growing season.

Seeds from eight half-sib sweetgum families were sown in the microplots during the third week of April. The experimental design was a factorial with 8 families, 4 fertilizer treatments, and 2 endomycorrhizal conditions. There were 40 planting locations in each microplot and each of the eight families were randomly assigned 5 of those locations. Four to six seeds were planted at each spot and the microplots were lightly covered with fumigated pine needle mulch. After germination, seedlings were thinned to one per planting spot.

The seedlings were harvested in mid-October. The presence or absence of infection by *G. mosseae* was determined from root samples from each microplot (2 seedlings/family) using the chloral hydrate acid-fuchsin clearing and staining procedure of Phillips and Hayman (1970). Heights and root collar diameters were measured, and root and top weights were obtained after drying to constant weight at 70°C.

Analysis of variance and Tukey's W-procedure (Steel and Torrie 1960) were used to identify significant responses. A mean separation test was also done on the pooled upland (families 2, 8, 9) and bottomland seed sources (families 4, 5, 6, 7, and 51).

RESULTS

Endomycorrhizal Effects.—Examination of the cleared and stained root samples from seedlings growing in plots containing *G. mosseae* showed that all were endomycorrhizal after 6 months and that control seedlings were nonmycorrhizal. The seedlings inoculated with *G. mosseae* outgrew the nonmycorrhizal ones (Fig. 1).
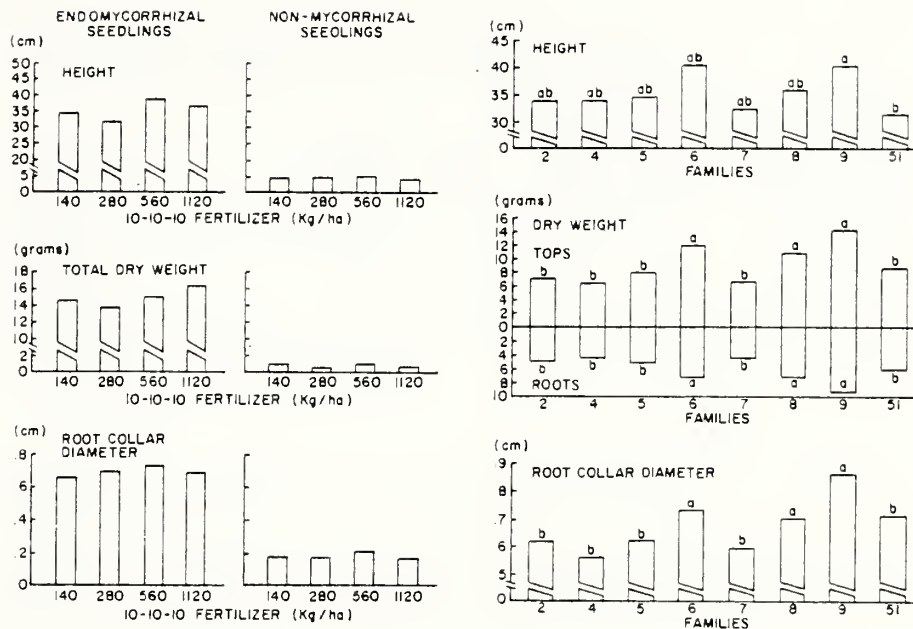
FIGURE 1. Pooled growth responses of endomycorrhizal and nonmycorrhizal sweetgum seedlings of eight families grown for 6 months in a soil-sand-bark mixture containing 140, 280, 560, and 1120 kg/ha of commercial 10-10-10 fertilizer. Bars for endomycorrhizal and nonmycorrhizal seedlings represent the means of approximately 120 and 50 seedlings, respectively.

FIGURE 2. Heights, dry weights, and root collar diameters of eight families of 6-month-old mycorrhizal sweetgum seedlings. Bars labeled with the same letter are not different at the 95 percent confidence level.

The values in Figure 1 are the pooled means of all families within fertilizer treatments. About 60 percent of all the nonmycorrhizal seedlings died during the growing season. This mortality and minimal growth of the surviving nonmycorrhizal seedlings precluded family comparisons between nonmycorrhizal and endomycorrhizal seedlings.

Fertilizer Effects.—Height growth was the only one of the six parameters tested that was significantly affected by fertilizer level (Table 1). Fertility level did not significantly influence growth of progeny from any of the eight mother trees.

Family and Ecotype Effects.—There were highly significant differences among families in growth of endomycorrhizal seedlings (Table 1, Fig. 2). Means of all growth parameters other than seedling height were significantly greater for the pooled upland families than for the bottomland families (Table 2).

DISCUSSION

The beneficial effects of the endomycorrhizal symbiont, *G. mosseae*, to sweetgum seedlings were not altered by high soil fertility. The endomycorrhizal seedlings grew equally well at the lowest and highest levels tested (Fig. 3). The nonmycorrhizal seedlings from all eight half-sib families failed to develop normally even with

TABLE 1. *Summary of* F-*tests from analyses of variance performed on sweetgum seedlings of eight families inoculated with* Glomus mosseae.

Source of variation	Seedling variable tested					
	Total weight	Root weight	Stem weight	Leaf weight	Root collar diameter	Height
Replication	**[1]	*[2]	*	**	NS[3]	**
Family	**	**	**	**	**	**
Fertilizer	NS	NS	NS	NS	NS	*
Family-fertilizer interaction	NS	NS	NS	NS	NS	NS

[1] ** *F* significant at 1 percent level.

[2] * *F* significant at 5 percent level.

[3] NS *F* not significant.

the application of 1120 kg/ha of 10-10-10 plus side dressings of NH_4NO_3 totaling 1680 kg/ha.

Most reports of plant responses to endomycorrhizal symbionts have been concerned with agronomic field or truck crops (Gerdemann 1968). Inoculation of these plants with endomycorrhizal fungi can increase growth and yield significantly. This increased productivity is greatest at low levels of soil fertility. In soils with optimum or higher fertility levels, nonmycorrhizal controls often develop as well as the endomycorrhizal plants (Gerdemann 1968). However, the greatest increase in growth by nonmycorrhizal plants occurs with the addition of high rates of phosphorus to the growing media. At the highest fertility level in this study, the nonmycorrhizal sweetgum seedlings had approximately 55–60 ppm of available P as well as adequate levels of the other elements. The concentration of P, although fairly high, was apparently not sufficient to alter the endomycorrhizal requirement of sweetgum. This lack of response by sweetgum to high levels of fertilization differs from that of most agronomic plants.

The uniform response of both the endomycorrhizal and nonmycorrhizal sweetgum seedlings to different fertility levels has practical applications in nursery management. The fertility levels tested approximate the normal range in forest tree nurseries growing hardwoods. The poor development of the nonmycorrhizal seedlings suggests that high fertility levels alone are not sufficient for good seedling production. However, even the lower and moderate rates applied to a naturally fertile soil may be adequate to grow sweetgum seedlings if endomycorrhizal symbionts are plentiful in the nursery soils. Unfortunately there is no information on the distribution and abundance of these essential root symbionts in nursery soils.

TABLE 2. *Growth parameters for pooled upland and bottomland families of mycorrhizal sweetgum seedlings.*[1]

Families	Total weight (gm)	Root weight (gm)	Stem weight (gm)	Leaf weight (gm)	Root collar diameter (cm)	Height (cm)
Upland	18.5	7.2	5.4	5.9	0.74	37.3
Bottomland	14.0	5.4	4.3	4.5	0.64	34.8

[1] Means from upland and bottomland families are significantly different for all growth parameters, except height, at the 5 percent level.
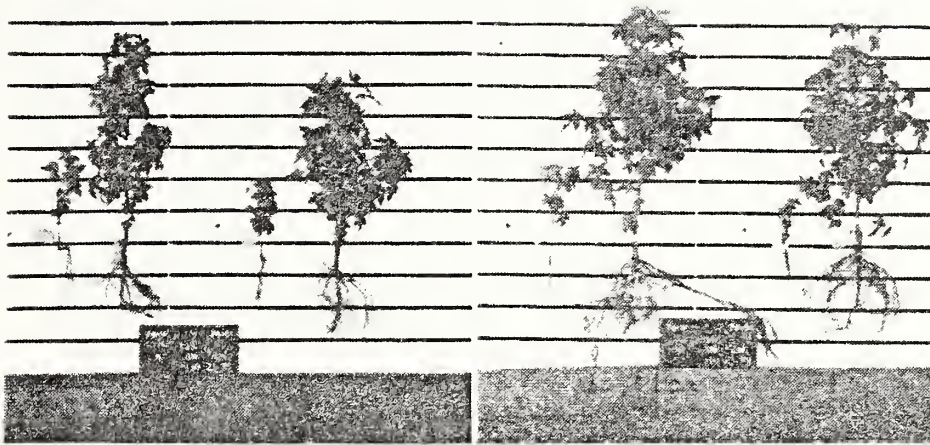
FIGURE 3. Left picture shows the responses of the best family (74-9) and the poorest family (74-51) to 140 kg/ha (125 lbs/a) of 10-10-10 fertilizer. Right picture shows the responses of the same two families to 1120 kg/ha (1000 lbs/a) of 10-10-10 fertilizer. For each family, a typical nonmycorrhizal seedling is at left, the smallest endomycorrhizal seedling is in the middle, and the largest endomycorrhizal seedling is at right.

This and earlier work (Bryan and Kormanik 1977, Bryan and Ruehle 1976) indicate that endomycorrhizae can have a significant effect on the size of sweetgum seedlings as well as reducing the number of substandard individuals. The erratic growth and consistent high percentage of substandard sweetgum seedlings produced in forest nurseries is apparently not from improper nutritional levels but from the lack of sufficient endomycorrhizal inoculum as a result of soil fumigation. Periodic fumigation is used in hardwood nurseries to control destructive pathogens and to control weeds. This practice can also destroy the endomycorrhizal inoculum in the zone of effective fumigation. Until the fumigated zone becomes naturally re-infested with viable inoculum or the roots grow below it and into the proximity of viable inoculum, sweetgum seedlings will not develop endomycorrhizae and may not develop properly. Since our control seedlings were not exposed to natural mid-season infection, because of thorough fumigation of the whole soil medium, they remained stunted throughout the season and did not exhibit late summer growth so commonly found in production nurseries. This absence of midseason infection may account for the high mortality observed here and earlier (Bryan and Kormanik 1977).

In addition to contributing to the enhancement of nutrient uptake, endomycorrhizae have also been shown to be beneficial in water uptake and transport by plants (Safir and others 1971, 1972). We observed that cessation of diurnal leaf wilting is an indicator of mycorrhizal formation. For 2 or 3 weeks after germination, the leaves of seedlings wilt during the day. Soon after the seedlings cease wilting, height growth normally commences and leaves expand rapidly. In the nonmycorrhizal plots, seedlings continue to exhibit diurnal wilting and leaves eventually turn red or purple.

Additional observations are required before much significance can be placed upon ecotypic adaptations to endomycorrhizal infection. The half-sib progeny from mother tree upland selections did grow significantly better than progeny from bottomland parents. However, except for the progeny from mother trees 4 and 7, both bottomland selections, the average root collar diameters of all families exceeded that

deemed necessary (0.763 cm) by Belanger and McAlpine (1975) for acceptable outplanting performance. These results and other work in progress indicate that it may be practical to evaluate selected mother trees as to their potential for producing a high percentage of plantable sized seedlings when nursery soils are infested with specific endomycorrhizal symbionts. It is not unreasonable to assume from these data that the significant ecotype response was nothing more than a mother tree response unrelated to planting site.

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